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THE RELATION BETWEEN AMINO-ACIDS AND THE WASSERMANN REACTION.

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AMONGST the large amount of work undertaken to explain the nature of the substance in syphilitic serum which is responsible for the positive Wassermann reaction, that of Mahlo aroused our interest. Mahlo (1913) claimed that he was able to make a Wassermann-negative serum positive by the addition to it, in a test-tube, of glycine in a dilution up to 1 in 1,000,000 and with leucine and tyrosine in a dilution up to 1 in 10,000. In a second series of experiments he claimed that the serum of rabbits which previously was Wassermann-negative was found to be strongly positive five hours and twenty-four hours after the injection of 5 c.c. tyrosine solution (1 in 50) intravenously, and that similar results were obtained after the injection of leucine solution and of the amino-acids obtained from a child's liver. Much and Schmidt (1921), while confirming Mahlo's results with regard to the injection of amino-acids into rabbits, were unable to confirm his test-tube experiments, and concluded that some process occurring in the body, in addition to the presence of amino-acid, is necessary for the production of a Wassermann-positive serum. Bachmann (1922) later carried out similar experiments and in general confirmed Mahlo's results. He also found that the results of the ninhydrin test (for amino-acids) with syphilitic sera agreed well with the results of the Wassermann reaction.

Little notice seems to have been taken of these results, which, however, if confirmed, are of considerable interest. Moreover the suggestion that the quantity of amino-acids in blood runs parallel with the results of the Wassermann reaction offers the possibility that the quantitative determination of amino-acids in blood might be an alternative or additional means of investigating the presence or absence of syphilitic disease.

We have therefore undertaken an investigation of the action of a number of amino-acids on Wassermann-negative sera both by direct addition *in vitro* and by injection into rabbits.

We have also carried out Wassermann reactions and blood amino-nitrogen determinations on specimens of blood obtained from patients attending the Venereal Diseases Clinic of the Leeds General Infirmary, and wish to record our results and observations on our experiments in the present communication. The results may conveniently be considered in two parts, viz. those dealing with the addition of amino-acids to serum either *in vitro* or by injection, and those dealing with the parallel determinations of Wassermann reaction and blood amino-nitrogen in hospital patients. These results are as follows:

I. ACTION OF AMINO-ACIDS ON BLOOD-SERUM.

(a) *Injection of Amino-acids into Rabbits.*

In this series of experiments various quantities of the different amino-acids mentioned were injected either intravenously or subcutaneously. In all cases blood was withdrawn and the Wassermann reaction carried out before the injection and at stated intervals subsequently, and in most cases amino-acid nitrogen determinations by the method of Folin (1922) were also made. These serve to show that in all cases the blood amino-acids were appreciably raised following the injections, and also show what concentration of amino-acid nitrogen was present in the blood when the Wassermann reactions were carried out.

Effect of Injection of Glycine.

Experiment 1.—A 1% solution of glycine in distilled water was made, the *p*H adjusted to 7·5 with N/10 NaOH, and 5 c.c., *i.e.* 0·05 gm., injected subcutaneously.

Wassermann reaction before injection . . .	Negative.
„ „ 5 hours after injection . .	„

Experiment 2.—2 gm. glycine were dissolved in 10 c.c. of distilled water and the solution adjusted and autoclaved. 7 c.c., *i.e.* 1·4 gm., injected.

	Amino-acid nitrogen (mgm. per 100 c.c. blood).	Wassermann reaction.
Before injection .	7·87 .	Negative.
6 hours after .	9·33 .	„
24 „ .	7·65 .	„
48 „ .	— .	„

Experiment 3.—4 gm. glycine in 20 c.c. distilled water injected intravenously.

	Amino-acid nitrogen.	Wassermann reaction.
Before injection .	8·0 .	Negative.
5 hours after .	12·3 .	„
24 „ .	6·4 .	„
48 „ .	7·4 .	„

Effect of Leucine.

Experiment 4.—1 gm. leucine dissolved in 50 c.c. of water injected subcutaneously.

	Amino-acid nitrogen.	Wassermann reaction.
Before injection	—	Negative.
1 hour after	11·3	,,
3 hours after	10·0	,,
5 ,,	8·0	,,
7 ,,	9·8	,,
23 ,,	8·6	,,

Effect of Arginine Base.

Experiment 5.—1·5 gm. in 10 c.c. distilled water injected intravenously.

	Amino-acid nitrogen.	Wassermann reaction.
Before injection	—	Negative.
1 hour after	12·4	,,
2 hours after	9·3	,,
4 ,,	8·6	,,

Effect of Arginine Nitrate.

Experiment 6.—1 gm. in 10 c.c. of distilled water injected intravenously.

	Wassermann reaction.
Before injection	Negative.
2 hours after	,,
4 ,,	,,
6 ,,	,,
24 ,,	,,

Effect of Taurine.

Experiment 7.—1 gm. in 10 c.c. of distilled water injected intravenously.

	Wassermann reaction.
Before injection	Negative.
1 hour after	,,
2 hours after	,,
4 ,,	,,
6 ,,	,,
18 ,,	,,

Since the effect of the single amino-acids so far used was found to be negative, it was decided to determine the effect of such protein decomposition products as are obtained by the hydrolysis of liver. For this purpose fresh human liver (200 gm.) was minced, 50 c.c. of water was added and 25 c.c. of trypsin solution (liquor pancreaticus-Benger). This mixture was incubated for different periods at 37° C., after which it was filtered, its reaction adjusted, and autoclaved.

The different fluids obtained will contain not only amino-acids, but certain

intermediate products of protein decomposition, and the relative proportions of the different substances present will vary according to the length of time the trypsin is allowed to act.

Experiment 8.—Fluid after 8 hours' trypsinization. 8 c.c. injected intravenously.

	Amino-acid nitrogen.	Wassermann reaction
Before injection	9·5	Negative.
1 hour after	11·4	,,
2 hours after	10·2	,,
3 ,,	9·3	,,
5 ,,	9·5	,,
7 ,,	9·6	,,
24 ,,	8·5	,,

Experiment 9.—Fluid after 24 hours' trypsinization. 5 c.c. injected intravenously and 5 c.c. subcutaneously.

Here a secondary rise in amino-acid nitrogen occurred, probably owing to the rate of absorption of the fluid injected subcutaneously being much slower than that injected intravenously.

	Amino-acid nitrogen.	Wassermann reaction.
Before injection	—	Negative.
1 hour after	11·8	,,
2 hours after	9·6	,,
4 ,,	11·7	,,
7½ ,,	11·2	,,
24 ,,	—	,,

Experiment 10.—Fluid after 48 hours' trypsinization. 10 c.c. injected intravenously.

	Amino-acid nitrogen.	Wassermann reaction.
Before injection	—	Negative.
1 hour after	12·7	,,
3 hours after	12·5	,,
5½ ,,	11·0	,,
22 ,,	8·7	,,

(b) *Direct Action* in vitro.

1% solution of glycine was prepared in each of the following ways:

(1) In normal saline solution.

(2) In normal saline solution, the *p*H being subsequently adjusted to *p*H 7·5 with N/10 NaOH.

(3) In distilled water.

(4) In distilled water with subsequent *p*H adjustment as in (2).

Quantities of each of these solutions were added to Wassermann-negative human sera to give resulting dilutions of glycine of 1 : 1,000,000, 1 : 100,000, 1 : 10,000 and 1 : 1000. Each of the resulting mixtures of serum and glycine solution were submitted to the Wassermann reaction. In every case the result was found to be negative.

Similar experiments were also carried out using leucine, arginine and taurine, again with entirely negative results.

In the next series of experiments the fluids resulting from trypsinization of liver, as described above in Experiments 8, 9 and 10, were each added to Wassermann-negative human sera in the proportion of 0·1 c.c. of the fluid to 1·5 c.c. of serum, and the Wassermann reaction of the mixtures determined. Again, the results were found to be negative.

Similarly the addition to Wassermann-negative sera of fluids obtained by heating a gelatine solution in an autoclave for 8 hours, and by hydrolysing gelatin by the action of sulphuric acid for 16 hours with subsequent neutralization, also failed to produce any change in the Wassermann reaction.

All these experiments having proved negative, it was decided to test the effect of the reverse procedure, namely, to withdraw certain amino-acids from a Wassermann-positive human serum and to determine the Wassermann reaction of the serum remaining and of the substances withdrawn. For this purpose normal butyl alcohol was used. Dakin (1918, 1919, 1920) found that normal butyl alcohol extracts the aliphatic and aromatic mono-amino-acids and proline practically quantitatively, but leaves behind the dicarboxylic acids—aspartic and glutamic—and the diamino-acids—histidine, lysine and arginine.

Accordingly 20 c.c. of serum whose Wassermann reaction was previously found to be positive were extracted *in vacuo* in an apparatus similar to that used by Dakin for 80 hours. At the end of this period the extracted serum was removed, any remaining butyl alcohol removed as far as possible by vacuum distillation, and the resulting fluid was submitted to the Wassermann reaction. The result was found to be positive. (It had been previously found that the presence of small amounts of butyl alcohol does not affect the Wassermann findings, so that any butyl alcohol still remaining in the serum after the treatment described could not have been responsible for the result obtained.) The butyl alcohol extract was evaporated almost to dryness by vacuum distillation, the residue thoroughly mixed with normal saline and submitted to the Wassermann reaction, when a negative result was obtained. The extraction was repeated in all six times with different Wassermann positive sera, with similar results in every case. A negative Wassermann serum was not altered by this treatment.

II. THE RELATION BETWEEN THE AMINO-ACID NITROGEN CONTENT OF BLOOD AND THE WASSERMANN REACTION.

In addition to the simultaneous determination of amino-acid nitrogen and of the Wassermann reaction of the blood of patients attending the venereal diseases clinic, a number of amino-acid nitrogen determinations were made on the blood of patients in the general wards of the hospital, suffering from various non-venereal conditions. The results obtained serve as controls for comparison with the results obtained in cases of syphilis. Further, a number of patients who after admission to the general wards were found to have a positive Wassermann reaction in the blood were included in the investigation. Of the cases dealt with at the Venereal Clinic, two were investigated at frequent intervals for a period of about four months, during which active anti-syphilitic

treatment was being carried out. Three cases of scarlet fever were also investigated, since it is claimed by some that the Wassermann reaction is occasionally found to be positive in patients suffering from that disease.

The results in these various classes of cases are as follows:

1. *Non-Venereal Cases in the General Wards.*

15 cases were examined. The blood amino-acid nitrogen was found to vary between 4·9 and 8·1 mgm. per 100 c.c., the mean being 6·46 mgm.

These results agree closely with the normal range of amino-acid nitrogen, viz. 5 to 8 mgm. quoted by other workers (*e. g.* de Wesselow, 1924).

2. *Cases of Syphilis in which Frequent Investigations were made over a Period of Treatment.*

(a) *Case 26/55.*

Date.	Amino-acid nitrogen.	Wassermann reaction.	Clinical notes.
19.1.26	7·03	Positive	Well-marked secondary rash; primary sore still present.
26.1.26	7·00	,,	—
2.2.26	7·78	,,	Rash appears stationary.
11.2.26	8·28	,,	Rash improving.
20.2.26	8·58	—	—
26.2.26	7·33	—	—
3.3.26	9·60	Negative	—
11.3.26	7·90	,,	—
18.3.26	7·87	,,	Rash considerably improved, only staining of skin visible.
14.4.26	4·88	,,	—
23.4.26	4·36	,,	—
30.4.26	6·76	,,	—
12.5.26	7·29	,,	—

(b) *Case 26/269.*

Date.	Amino-acid nitrogen.	Wassermann reaction.	Clinical notes.
29.3.26	4·42	—	Secondary rash; primary sore still present.
7.4.26	5·45	Positive	Clinical condition followed a normal course.
14.4.26	4·24	Doubtful	
23.4.26	6·64	,,	
4.5.26	3·89	Slight positive	
11.5.56	5·90	Negative	
18.5.26	6·37	,,	
1.6.26	7·07	,,	
9.6.26	9·65	Anti-complementary	

3. *Other Cases Attending Venereal Clinic.*

(a) *Non-syphilitic cases, including gonorrhœa, non-venereal urethritis, balanitis, herpes genitalis.*

Of 10 cases, all having a negative Wassermann reaction, the amino-acid nitrogen ranged from 5·2 mgm. to 9·3 mgm., the mean being 6·3 mgm.

(b) *Syphilitic cases.*

(i) Primary without treatment.

Case.	Amino-acid nitrogen.	Wassermann reaction.	Clinical notes.
26/61	5·60	Positive	Balanitis and phimosis.
26/262	7·00	Negative	Early primary disease.

(ii) Secondary without treatment.

Case.	Amino-acid nitrogen.	Wassermann reaction.	Clinical notes.
26/246	7·25	Positive	Late secondary.

(iii) Treated cases.

Outstanding cases bearing on the relation between amino-acid nitrogen and Wassermann reaction are:

Date.	Case.	Amino-acid nitrogen.	Wassermann reaction.	Clinical notes.
29.1.26	5637	7·11	Slight positive	Old syphilis (13 years), scarring of soft palate. Admitted 4.3.20, when Wassermann reaction positive.
2.2.26	10045	6·83	„	History extending over 3 years before admission in 1922. Had much treatment.
17.2.26	6459	7·37	Doubtful	Old syphilis with glossitis, Wassermann reaction has varied between negative and doubtful.
18.5.26	5165	7·11	Positive	Secondary syphilis in 1920. Wassermann reaction negative until this date.
21.6.26	11282	8·54	„	Admitted May, 1923, with gonorrhœa. Syphilis also found.
21.6.26	24/187	8·24	„	First treated in 1919 for primary disease; defaulted, then re-attended.
21.6.26	25/951A	8·19	„	Old syphilis, now affecting central nervous system.
24.6.26	25/529	6·17	„	Syphilis 16–17 years ago.
24.6.26	25/973A	5·07	„	Admitted 1925 with gumma of thigh. History of gonorrhœa 30 years ago.

In addition 15 cases having a negative Wassermann reaction showed amino-acid nitrogen values between 7·73 and 5·13.

4. *Cases with Positive Wassermann Reaction in the General Wards.*

9 cases were examined, having amino-acid nitrogen values varying between 7·78 and 5·39.

5. *Cases of Scarlet Fever.*

3 cases, all showing negative Wassermann reactions, gave amino-acid nitrogen values of 6·83, 6·97 and 7·86.

An examination of the foregoing results shows clearly that no close relationship exists between the amino-acid nitrogen content of the blood and its Wassermann reaction, thus confirming the results of our experiments *in vitro* and on rabbits. A certain number of cases with a positive Wassermann reaction do show high normal or definitely raised amino-acid values, but the number of cases in which a positive Wassermann reaction co-exists with a normal or low amino-acid value, or a negative Wassermann (both in syphilitics and in non-syphilitics) co-exists with a high normal or raised amino-acid value shows conclusively that the two findings are not interdependent, and that amino-acid nitrogen determinations are valueless as a means of investigating the presence or absence of syphilitic disease. In the two cases investigated over a period, considerable variations in the amino-acid content of the blood were found, but in neither case was the variation found to run parallel with the variation in the results of the Wassermann reaction. Since Wassermann reaction and amino-acid both vary over a period, it will be possible in many cases, owing to coincidence, to find a positive Wassermann reaction and high amino-acid in the same sample of blood, and this is probably the only reason why the two are found to co-exist.

CONCLUSIONS.

In experiments *in vitro* and on rabbits, and in parallel determinations of blood amino-acid nitrogen and Wassermann reaction in a number of hospital patients, both syphilitic and non-syphilitic, we have failed to demonstrate any relation between the amino-acid content of blood and its Wassermann reaction.

We wish to express our sincere thanks to Dr. J. P. Bibby, physician to the Venereal Diseases Clinic of the Leeds General Infirmary, for his very valuable co-operation and help in this investigation, and to the Medical Research Council for a grant-in-aid to one of us (J. G.).

REFERENCES.

Bachmann, W.—(1922) *Zeitschr. f. Immunitätsforsch.* (Orig.), **33**, 233.
Dakin, H. D.—(1918) *Biochem. J.*, **12**, 290.—(1919) *Ibid.*, **13**, 398.—(1920) *J. Biol. Chem.*, **44**, 499.
De Wesselow, O. L. V.—(1924) 'The Chemistry of the Blood in Clinical Medicine, (Benn), London.
Folin, O.—(1922) *J. Biol. Chem.*, **51**, 377.
Mahlo.—(1913) quoted by Bachmann.
Much, H., and Schmidt, H.—(1921) *Deutsch. med. Woch.*, **47**, 552.

THE LIPOLYTIC ACTIVITY OF TUBERCULAR GUINEA-PIGS' TISSUES.—I.

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DURING the past four years attention has been drawn to the activity of the various enzymes in different tissues, and studies have been made concerning the way in which the activity of the lipases and proteases of normal animals is affected by disease. Thus Falk, Noyes and Sugiura (1924) have studied the enzymic activity of rat carcinoma, normal rat tissues, and corresponding tissues from animals suffering from the Flexner-Jobling rat carcinoma.

In 1925 Capelli made the statement that the tubercle bacillus was disintegrated by lipase (source not stated)—a suggestion which, although of great interest, is yet open to some doubt on the evidence given by him, since it is extremely difficult to free lipase from proteolytic enzymes. Proteolytic enzymes might easily have accounted for the disintegration of the bacilli which he observed.

Bulloch and McLeod (1904) showed that by alcoholic extraction of the tubercle bacillus it was possible to obtain a fatty mixture from which oleic, isocetinic, myristic and lauric acids were probably obtained. The acid-fast nature of the bacillus did not, however, appear to be associated with these substances, but with a waxy substance proved to be a higher alcohol.

The inability to destroy this capsular acid-fast material has repeatedly been urged as the reason for man's frequent failure to destroy the tubercle bacillus, once it has lodged in certain body tissues.

This raises the question whether enzymes which are capable of effecting the destruction of the bacillus exist in the body. If we suppose that the acid-fast higher alcohol is combined in ester form with one of the fatty acids, it might be that a disintegration of the bacillus could be effected by some or all of the body lipases.

Also, if we consider the hypothesis that entry of the tubercle bacillus into susceptible body tissues must often occur without resulting infection, the possibility of some difference in the lipolytic activity of the comparatively immune person's tissue as compared with that of the susceptible person must be visualized. Whether there is any inter-relationship between tubercle infection and lipolytic activity seemed to us, therefore, worthy of study.

With this possibility in view, we have studied initially the lipolytic activity of lung, liver, heart, spleen and kidney tissues of normal and tubercular guinea-pigs to ethyl butyrate, and in order to compare the results with those of animals suffering from an exotoxæmia a few diphtheritic animals have also been used.

It has previously been determined that the rate of hydrolysis of an ester under the action of a lipase in the presence of an excess of the substrate is directly proportional to the concentration of the lipase. (Platt and Dawson, 1925; Thomson and Meleney, 1924.)

These results we have confirmed by a method similar to that of Thomson and Meleney, noting the time taken for a lipase preparation acting upon 0·2 c.c. ethyl butyrate (predetermined excess of the substrate) to effect a change from *p*H 8 to *p*H 7·2 at a temperature of 37·5° C., the intermediate value *p*H 7·6 also being noted. These times were plotted against the amount of enzyme taken. It was noticed that the graph was linear, except at extremely small concentrations of the enzyme preparation used, and that it was unaffected by taking different amounts of the substrate in excess of 0·2 c.c.

EXPERIMENTAL.

Normal diphtheritic or tubercle-infected guinea-pigs were killed by bleeding, and the heart, lung, liver, spleen and kidneys were carefully removed and freed from gross fat. The organs were weighed, immediately placed in water under toluene and transferred to the Royal Technical College, Salford. They were then ground up with sand, washed into a stoppered bottle, and extracted with a 50% glycerine-water mixture, the following quantities being used for the different organs: Liver, 1 or 2 litres; lung and kidneys, 500 to 700 c.c.; heart, 250 to 500 c.c.; spleen, 20 to 250 c.c., according to the weight and condition of the organs. These quantities were found to be adequate for the extraction of about 98% of the lipase effecting the hydrolysis of ethyl butyrate. The bottle was tightly stoppered and placed in the ice-chest for 48 hours. The extract was strained through a coarse straining-cloth fitted into a Buchner funnel and thoroughly washed through; the filtrate and washings were made up to a suitable volume with distilled water, placed under toluene, tightly stoppered and kept in the ice-chest until required.

Estimation of lipolytic activity of liver, lungs and kidneys.—Three 50 c.c. flat-bottomed Jena flasks were taken, one as the reaction vessel and the other two as controls. To the reaction flasks were added 10 c.c. of Clark's phosphate buffer mixture *p*H 8, a suitable volume of the extract and a suitable amount of a 0·2% solution of phenol red. To the control vessels were added: (1) 10c.c. phosphate buffer, *p*H 7·6, a volume of boiled enzyme extract equal to that used in the experimental flask, the same amount of phenol red and 0·2 c.c. of ethyl butyrate. (2) 10c.c. phosphate buffer solution *p*H 7·2, boiled enzyme, phenol red and ethyl butyrate as in (1). The three flasks, tightly stoppered, were immersed in a thermostat regulated at 37·5°C. When the contents had reached the temperature of the bath 0·2 c.c. of ethyl butyrate was added to the reaction vessel.

The flasks were shaken every half minute and the time taken for the reaction vessel to reach *p*H 7·2 noted. Comparisons were always made under a standard daylight lamp.

The time taken for the reaction vessel to reach *p*H 7·6 was also noted, but for comparative results the total time taken to complete the reaction *p*H 8·0 to *p*H 7·2 was used.

Duplicate readings were always taken and usually agreed to within half a

minute. The mean of the two values is always presented in the following tables:

Estimation of lipolytic activity of heart and spleen tissues.—In these organs the lipase activity is so small that the technique had to be modified and 0·5 c.c. of the buffer solution was used instead of the previous 10 c.c., the difference in volume being made up with water, double distilled from glass.

The *p*H 7·6 reading is also omitted.

In order to check the success of the extraction process, a liver, weight 29·2 gm., was ground up, extracted with 500 c.c. of the 50% glycerol water-mixture, and made up to 1000 c.c. with the same mixture as previously described.

With 0·5 c.c. of the preparation, 15 minutes were required for the hydrolysis of the ethyl butyrate to liberate enough acid to change the *p*H value of the system to its final reading. Re-extraction of the tissue with the same quantities of extractant gave a complete reaction in 8 minutes per 10 c.c. of the new preparation, so that there was approximately a 90% extraction.

When this was repeated, and in all cases the extractant liquids doubled, approximately 98% extraction resulted. A third extraction revealed no further demonstrable activity.

As a further test of the completeness of the extraction, a sample of pig's liver was taken and divided into three portions, each of which was weighed. One of the authors was then given the weight of the one portion and asked to estimate the weights of the other two on a basis of comparative activity. The following results were obtained:

Actual weight of tissue.	Calculated weight.
11·2 gm. . . .	11·2 gm.
17·00 „ . . .	16·05 „
20·23 „ . . .	21·11 „

The extraction method is thus accurate to approximately ± 5%. To test the stability of the preparation during the periods of experimentation, several preparations were analysed on each alternate day for 14 days. Identical readings were always obtained. The tubercle-infected animals were all infected with tubercle bacilli contained in milk, the sediment from the centrifuged milk being used for the injections, which were given into the left hind leg of the animal. The diphtheritic animals were some which had been used for virulence tests on freshly isolated organisms from the human throat. They were killed, when suffering from a severe toxæmia, twenty hours after inoculation.

The comparisons of lipolytic activity are made by calculating the time taken to effect the standard change on a fixed weight basis, *e.g.* per 0·025 gm. of tissue in the case of the liver. Experiments are now in progress to test the accuracy of the results obtained working on a fixed total nitrogen basis.

RESULTS.

Reaction-Times of Normal Animal Tissues.

Twelve animals taken, two of which were males. Value based on constant weight of tissue: livers per 0·025 gm. tissue; lungs, 0·25 gm.; kidneys, 0·20 gm.; hearts, 0·25 gm.; and spleen, 0·25 gm.

Livers.—Average value, 12·3 mins.; range 8·8 to 15·9 mins.

Lungs.—Average value, 16·3 mins.; eleven animals range between 12·0 and 21·8 mins.; one male gives value of 29·2 mins.

Kidneys.—Average value, 17·7 mins.; nine animals range between 12·4 and 18·3, three animals give values of 24·1, 27·9 and 28·9 mins.

Hearts.—Average value, 19·9 mins.; range, 11·9 to 26·7 mins.

Spleens.—Average value 21·9 mins.; range, 18·0 to 35·0 mins.

Reaction-Times of Tuberculous Animals.

Fifteen animals taken, five of which were males.

Livers.—Average value, 22·8; uniform range from 12·1 to 48·8 mins.

Lungs.—Average value, 17·6 mins.; two animals give values of 7·9 and 9·8 mins.; twelve animals range between 13·7 and 23·5 mins.; one animal gives a value of 34·4 mins.

Kidneys.—Average value, 23·9 mins.; uniform range of 15·9 to 41·6 mins.

Hearts.—Average value, 28·4 mins.; two animals give values of 5·3 and 14·5 mins., remainder range between 23·2 and 41·0 mins.

Spleens.—Average value, 39·9 mins.; uneven groupings between 12·9 and 108·0 mins.

Reaction-Times of Diphtheritic Animals.

Three diphtheritic animals gave values as below:

Livers.—11·5 to 12·6 mins.

Lungs.—32·5, 42·7 and 43·0 mins.

Kidneys.—39·5, 52 and 74 mins.

Hearts.—7·2, 17·6 and 22·0 mins.

DISCUSSION.

A comparison made between the average values and the range of the reaction-times of normal and tubercular guinea-pig tissues reveals a markedly slower reaction with the liver, kidney, heart and spleen tissues of the tubercular animals, while the lung tissues yield such slight differences that one might almost say that they are uniform.

In the experiments as performed, the spleen, liver and lungs were affected by tubercle formation; the two former were usually riddled with coarse tubercles.

It was also noticed during the course of this work that the slowest reaction-times obtained from the affected organs were those in which coarse tubercular granulation was at a maximum.

The depressed lipolytic activity of the tubercular heart and kidney tissues, tissues not actively invaded by the bacillus, suggests that there is present in the system a general toxæmia which weakens the activity of the various organ lipases.

The hypo-activity of the tubercular livers and spleens might then be due to two causes:

(1) The large amount of comparatively inactive tubercular granulation-tissue present.

(2) A depressed activity caused by a general toxæmia.

The presence of tubercular granulation-tissue in the spleens, livers and lungs would, however, make the reaction-times of the functioning tissue of these organs appear slower than they actually were, since the activity is estimated on a total weight basis, a value necessarily containing the weight of the comparatively inactive granulation-tissue.

These facts make a deduction concerning the results obtained with the livers and spleens, organs actively attacked by the tubercle bacillus, in comparison with those of the hearts and kidneys of the tubercular animals, impossible. This, however, does not apply to the results obtained from the lungs.

If the depressed reaction-times of the heart and kidney tissues in the tubercular animals is due to a general toxæmia, why should the results obtained with the tubercular lungs be approximately normal?

Two hypotheses suggest themselves.

It might be that in those organs where there is an actual reaction between the bacillus and the organ tissue, there is a stimulation of lipolytic activity in an attempt to effect the destruction of the invader by the breakdown of the acid-fast material of the bacillus. It might equally be possible that a stimulus does occur in these organs which affects, not only the lipases of the organs, but the other enzymes, in an attempt to supply the normal body functions of the organs.

SUMMARY.

(1) A technique for the estimation of the lipolytic activity of tissue preparations is described.

(2) The lipolytic activity of normal tubercle-infected and some diphtheritic guinea-pigs' livers, lungs, spleens, hearts and kidneys have been compared.

(3) The normality of the lung results of tubercular animals suggests that in view of tubercle formation, the rest of the lung-tissue may be hyperactive.

We should like to mention that the organs used have been controlled in the majority of cases histologically, and we would acknowledge our indebtedness to Dr. Wm. Susman, of the Department of Pathology of this University, for his kindly assistance in the work.

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Note.—The animals used throughout have been used under the licence of the Public Health Department, York Place, Manchester, and were those used for the routine work of the Department.

REFERENCES.

Bulloch, W., and McLeod, J. J. R.—(1904) *J. Hyg.*, **4**, 1.
Capelli, G.—(1925) *Giorn. di med. milit.*, **4**.
Falk, G. K., Noyes, H. M., and Sugiura, K.—(1924) *J. Biol. Chem.*, **59**, 183.
Platt, B. S., and Dawson, E. R.—(1925) *Biochem. J.*, **19**, 860.
Thomson, W. P., and Meleney, F. L.—(1924) *J. Exp. Med.*, **40**, 233.

THE LIPOLYTIC ACTIVITY OF TUBERCULAR GUINEA-PIGS' TISSUES.—II: THE RELATIONSHIP OF CELL AND LIPOLYTIC ACTIVITY IN DISEASE.

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IN the preliminary experiments of this investigation reported in the previous paper, the biochemical examination revealed a lessened lipase activity in the organs of tubercular animals. Whether this is a general characteristic of a particular disease, or a result depending on the reaction of the cell to any disease process, is uncertain. It seemed, however, that a histological examination of the tissues from experimental animals might offer a solution to the problem.

TECHNIQUE.

The tissues for examination were removed from representative parts of the organs and placed in fixative within a few minutes after the animal was killed. Fixative: Helly's fluid. Embedding: Paraffin method. Stains: (1) Hemalum and eosin; (2) acid fuchsin and methyl green.

SUMMARY OF THE HISTOLOGICAL EXAMINATION.

In considering the histological descriptions of the tubercular and diphtheritic organs, the following should be kept in mind. The average lipase reaction-times of the normal animals were: livers, 12·3 mins.; lungs, 16·3 mins.; kidneys, 17·7 mins.; hearts, 19·9 mins.; spleens, 21·9 mins. The reaction-times of the organs described are given in brackets.

Series I. Tubercular Guinea-Pigs.

No. 6: *Liver* (reaction-time = 13·4 mins.), extensive early fatty degeneration, numerous small cellular tubercles. *Lung* (13·9 mins.), very few early tubercles. *Kidney* (15·9 mins.), somewhat congested. *Heart* (23·2 mins.), granular and some fatty degeneration. *Spleen* (37·3 mins.), mainly tuberculous granulation tissue; otherwise considerable hyperplasia: an appreciable amount of normal tissue.

No 7: *Liver* (12·1 mins.), few cellular tubercles. *Lung* (14·5 mins.), few isolated tubercles. *Kidney* (19·4 mins.), cloudy swelling. *Heart* (33·0 mins.), atrophy, granular degeneration. *Spleen* (17·5 mins.), numerous extensive tubercles, some hyperplasia; normal tissue appreciable.

No. 8: *Liver* (13·7 mins.), a number of large tubercles; fatty degeneration; normal tissue abundant. *Lung* (9·8 mins.), few tubercles; inter-alveolar

hyperplasia. *Kidney* (16·25 mins.), extensive acute glomerular and interstitial changes. *Heart* (36·0 mins.), degeneration and fibrosis. *Spleen* (34·1 mins.), very extensively tuberculous.

No. 9: *Liver* (18·8 mins.), a few tubercles; some fatty degeneration. *Lung* (15·1 mins.), inter-alveolar hyperplasia. *Kidney* (30·0 mins.), slight congestion. *Heart* (41·0 mins.), fibrosis; degeneration—granular and fatty. *Spleen* (108 mins.), very extensively tuberculous granulation-tissue; some hyperplasia.

No. 10: *Liver* (19·4 mins.), many tubercles, but largely healthy. *Lung* (21·6 mins.), a few cellular tubercles; congestion and hæmorrhage. *Kidney* (21·9 mins.), tubercular degeneration. *Heart* (25·0 mins.), granular and hyaline degeneration. *Spleen* (78·0 mins.), chiefly caseating tubercles.

No. 14: *Liver* (30·2 mins.), largely tuberculous granulation-tissue. *Lung* (14·9 mins.), a few cellular tubercles and some acute broncho-pneumonia. *Kidney* (17·4 mins.), a few small tubercles.

No. 15: *Liver* (18·5 mins.), fatty degeneration; several caseous tubercles. *Lung* (13·7 mins.), inter-alveolar hyperplasia. *Kidney* (19·4 mins.), some glomerular and tubular damage; general active congestion. *Heart* (38·9 mins.), fibrosis, degeneration—granular and fatty. *Spleen* (24·4 mins.), extensive cellular tuberculous granulation-tissue.

Series II. Diphtheritic Guinea-pigs.

No. 1: *Liver* (12·6 mins.), some fatty change. *Lung* (42·7 mins.), acute broncho-pneumonia. *Kidney* (39·5 mins.), hæmorrhage and necrosis. *Heart* (22·0 mins.), some granular and hyaline degeneration.

No. 2: *Liver* (11·5 mins.), nothing abnormal. *Lung* (43·0 mins.), acute broncho-pneumonia. *Kidney* (52·0 mins.), extensive early tubular degeneration. *Heart* (17·6 mins.), nothing abnormal.

No. 3: *Liver*, fatty degeneration. *Lung* (32·5 mins.), congestion and hæmorrhage. *Kidney* (74·0 mins.), extensive tubular degeneration. *Heart* (7·5 mins.), very active congestion. *Spleen* (53·0 mins.), extensive degeneration; very little normal tissue.

DISCUSSION.

Even a cursory survey discloses a grossly irregular lipase reaction-time in the corresponding organs of the experimental animals. As the animals had been inoculated by similar methods, and as the time which elapsed between the inoculation and death did not vary greatly, the reason must be sought in another direction. That there is no uniform decrease in reaction for any particular organ in tuberculosis is evident.

Perhaps the fact that some organs gave a very slow reaction-time may suggest a solution. These organs are invariably badly damaged. It is evident, too, that if there has been any extensive proliferation of the cells of that organ, *e. g.* hyperplasia in the spleen, the lipase reaction-time is above that of cellular tubercles, but considerably below that of normal tissue. Organs which exhibit extensive necrosis are the slowest in reaction.

Other causes must be found, however, to explain many of the low reaction-times obtained in this investigation. The lungs suggest that in collapse, in

emphysema and in the presence of hæmorrhage or œdema over any considerable area, the lipase reaction-time may be below the average. Since both blood and the body-fluids contain a lipolytic enzyme, it is neither their presence nor their absence which can explain the change. The common feature is that the organ over extensive areas has become largely functionless. That loss of function can reduce the lipase-reaction of the involved cells is reasonable and consistent with the findings.

In only a few organs was there any degree of increased activity of the lipase. When this occurred there was a general picture of increased cell-activity (compatible with a pre-inflammatory condition).

Although these features have been discussed separately, they seldom occurred singly. For this reason, it is essential to make a collective estimate on all the changes present in that organ. For example, an organ in extensive necrosis may give a moderate reaction-time. In such a case there may be regeneration, or over areas of appreciable extent pre-inflammatory cellular activity may be pronounced. The latter may counterbalance considerably the depression caused by necrosis. The necessity for a collective estimate of the condition of the organ is exemplified in the tubercular lungs, whereas in the lungs of the diphtheritic animals the changes present are so extensive that they clearly indicate the relationship between lipolytic and cell-activity. In the tubercular animals pulmonary lesions are not severe. Although tubercles occur they are cellular, and the inflammatory reaction in the remaining tissue is not as yet of a destructive but of a stimulating nature. Hence there has been some degree of hyperplasia of the cells of the alveolar walls. In other words, cell-production is the prominent feature and cell-destruction is at a minimum.

In these circumstances, it can readily be expected that even a moderate degree of damage, such as a few areas of broncho-pneumonia, or perhaps atrophic emphysema, will make little if any impression on the lipolytic reaction-time. This was particularly the case in the lungs of the tubercular animals.

These results, when examined in conjunction with the essentially biochemical results presented by Happold and Taylor (1927), while not disposing of the possible hypothesis there mentioned, that is, the specific importance of the relationship of lipolytic function and tubercular infection, yet would suggest a probable greater importance to the other hypothesis advanced there and here—the hypothesis that the reaction between the invading organism and the susceptible tissue-cells, leading as it does to necrosis of the tissue, calls forth an increased functional activity of the undestroyed tissue. This increased function is due either to multiplication of that tissue by rapid cell-division, or to increased function of the tissue itself, in an attempt to supply the normal demands made by the animal, or probably to both.

If this were so, we would expect to find not only an increased lipolytic activity, but an increased proteolytic or other enzymic activity in the organs concerned.

Further experiments are being undertaken in order to establish the true position as between the hypotheses postulated.

In conclusion, the authors would like to express their indebtedness to

Mr. Albert Taylor, of the Royal Technical College, Salford, for assistance in the estimation of the lipolytic activities of the tissues examined.

SUMMARY.

1. Proliferation of the functioning cells of an organ causes an increase in its lipolytic activity.

2. Necrosis, on the contrary, establishes a decrease which is proportional with the extent of the lesion. This is also true when cells, although still intact, become functionless.

3. Proliferation of stroma causes a decrease, but to a lesser degree than necrosis.

4. The reaction varies according to the damage to the cells of the organ and not according to the disease.

REFERENCE.

HAPPOLD, F. C., AND TAYLOR, A.—(1927) *Brit. J. Exp. Pathol.*, **8**, 101.

ON THE BACTERICIDAL ACTION OF MERCUROCHROME.

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"MERCUROCHROME 220 soluble" is the name given to a compound of mercury with dibrom-fluorescein, to whose "antiseptic" properties attention was first called by Young, White and Swartz (1919). Finding that this drug in dilute solution in urine would kill microbes rapidly—as many other "antiseptics" will do—these authors recommended its direct instillation into the bladder and kidney pelves for the treatment of urinary infections.

Later papers, Hill and Colston (1923) and Hill and Scott (1925) reported that the drug when administered intravenously to rabbits sometimes conferred some slight bacteriostatic power upon the blood for an hour or two after the injection, and that it was excreted into the urine and the bile, making both these fluids bactericidal. Hill and Scott (1925) stated further that experimentally induced typhoid infections of the gall-bladder of rabbits were completely and rapidly cured by intravenous or oral administration of the drug.

* Working with a whole-time grant from the Medical Research Council.

On the basis of these reports, Young, Hill and Scott (1925) have recommended the intravenous injection of mercurochrome as an "internal disinfectant" in cases of septicæmia and severe localized sepsis. Its employment for the treatment of typhoid carriers has also been suggested. Some hundreds of cases of septic infection have now been reported upon. Striking clinical successes are claimed by some writers; others emphasize the severe toxic effects of the drug.

Recently, however, the publication of further laboratory work in connection with mercurochrome has thrown some doubt upon the earlier conclusions with regard to its alleged bactericidal efficiency *in vivo*. Walker (1926) has found that additions of mercurochrome, ranging from 1 part in 25,000 to 1 in 400, to human blood definitely favours rather than hinders the growth of staphylococci and streptococci in that blood; while the power of the blood to kill *B. coli* was unaffected by such additions. Further, Meyer, Sommer and Eddie (1926) have found that the intravenous injection of the drug into rabbits confers only an insignificant bactericidal power upon the bile, and they failed to cure experimental gall-bladder infections (*B. typhosus*) in these animals.

In view of the discrepancy between these results and those previously mentioned, the following experiments have been carried out.

IN VITRO EXPERIMENTS.

Experiment 1.—Graduated dilutions of mercurochrome* were made in distilled water, and of these, in each case, 1 part was added to 9 parts of defibrinated human blood. After one hour at room temperature the bloods were centrifuged and the sera pipetted off. The bactericidal power of these sera was then determined, staphylococcus and hæmolytic streptococcus being used as the test organisms. The test was carried out by incubating in capillary pipettes 25 c.mm. volumes of each serum with 5 c.mm. volumes of a series of dilutions of the microbic culture, and subsequently explanting these infected sera into melted agar in a Petri dish.

The number of colonies which developed is shown in Tables I and II:

TABLE I.

Culture used for the test: *Staphylococcus aureus*. Period of contact of the sera and cocci before explanting: 5 hours.

	Dilution of the broth culture.	
	1 in 900.	1 in 2700.
Number of staphylococci implanted in each volume of serum	(357)†	119
Number of colonies which grew from—		
Serum of control blood (no mercurochrome)	4064	3520
„ blood containing mercurochrome 1 in 40,000	6400	4192
„ „ „ „ 1 in 20,000	—	3200
„ „ „ „ 1 in 10,000	5664	2916

* The mercurochrome which we have employed for these experiments was supplied by Messrs. Martindale & Co.

† The numbers shown in brackets, in this and subsequent tables, represent counts obtained by calculation from the actual figures obtained on planting out a measured volume of the weaker dilution.

TABLE II.

Culture used for the test: a hæmolytic streptococcus. Period of contact of sera and cocci before explanting: 16 hours.

	Dilution of the broth culture.	
	1 in 1000.	1 in 10,000.
Number of streptococci implanted in each volume of serum	(320)	32
Number of colonies which grew from—		
Serum of control blood (no mercurochrome)	—	∞
„ blood containing mercurochrome 1 in 40,000	∞	∞
„ „ „ „ 1 in 20,000	∞	∞
„ „ „ „ 1 in 10,000	∞	∞

Experiment 2.—Samples of defibrinated human blood were mixed with mercurochrome as in Experiment 1. Each sample was then infected with staphylococci, and its power to kill these microbes determined by "inculturing" in "slide-cells" as described by Wright, Colebrook and Storer (1923).

The number of colonies which grew in the blood is shown in Tables III and IV:

TABLE III.

	Dilution of the broth culture.	
	1 in 1200.	1 in 3600.
Number of staphylococci implanted in each 50 c.mm. sample of blood	(180)	60
Number of colonies which grew in—		
The control blood	16	6
Blood containing 1 part of mercurochrome in 10,000	69	44

TABLE IV.

	Dilution of the broth culture.	
	1 in 1200.	1 in 3600.
Number of staphylococci implanted in each 50 c.mm. volume of blood	(159)	53
Number of colonies which grew in—		
The control blood	2	1
Blood containing mercurochrome 1 in 20,000	7	4
„ „ „ 1 in 40,000	7	3
„ „ „ 1 in 80,000	6	4

These results show: (1) That the addition of mercurochrome to human defibrinated blood *in vitro* conferred no bactericidal power to staphylococcus

and hæmolytic streptococcus, either upon the blood fluids or upon the blood as a whole; (2) that the bactericidal effect of normal human blood upon staphylococcus is definitely impaired by the addition to it of 1 part in 10,000 of mercurochrome. This reduction is no doubt due to an injurious effect of the drug upon the leucocytes—an effect comparable to that which our colleague, Dr. A. Fleming (1924), has shown to occur when most of the common "antiseptics" are added to blood *in vitro*.

IN VIVO EXPERIMENTS.

Bactericidal Tests of the Blood of Rabbits following the Injection of Mercurochrome.

Experiment 3.—Samples of blood were drawn from a rabbit before and at intervals after an intravenous injection of mercurochrome (5 mgm. per kilo). After separation of the serum from each of these samples a bactericidal test was carried out as in Experiment 1 *supra* by the explantation method.

TABLE V.

Culture used for the test: a hæmolytic streptococcus. Period of contact of the sera and cocci before explanting: 16 hours.

	Dilution of the broth culture employed.	
	1 in 1000.	1 in 10,000.
Number of streptococci implanted in each volume of serum	168	16
Number of colonies which grew from—		
Serum taken before mercurochrome	∞	∞
* ,, ,, 15 minutes after	∞	∞
,, ,, 2 hours after	∞	∞

This result shows that the animal's serum, even 15 minutes after the injection of the drug, had no appreciable bactericidal or bacteriostatic power for hæmolytic streptococci.

The bactericidal power of the defibrinated blood of rabbits before and after an intravenous dose of the drug, when tested in slide-cells, shows variations of the same magnitude as that from an untreated rabbit tested at the same time.

Bactericidal Tests of Bile following an Injection of Mercurochrome.

Experiment 4.—Blood was drawn from a rabbit before an intravenous injection of mercurochrome (5 mgm. per kilo). Half an hour later a second specimen of blood was taken, the rabbit killed and bile immediately collected from the gall-bladder. A control specimen of bile was collected from a second (untreated) rabbit. Serum obtained from the two samples of blood and also

* This serum had a decidedly pink tinge.

the bile specimens from the normal and the treated animals were now tested with respect to their power to kill *B. typhosus* (Table VI). The reservoir-pipette technique introduced by Sir Almroth Wright for this purpose was employed (*vide* Wright and Colebrook, 1921).

TABLE VI.

Dilution of the typhoid culture employed.	Number of typhoid bacilli implanted in each volume of serum.	Serum taken before injection of mercurochrome.	Serum taken ½ hour after.	Bile of untreated rabbit.	Bile of treated rabbit.
1 in 5	(37,500)	Growth	Growth	Growth	Growth
1 in 25	(7500)	,,	,,	,,	,,
1 in 125	(1500)	,,	,,	,,	,,
1 in 625	(300)	Sterile	,,	,,	,,
1 in 3125	(60)	,,	Sterile	,,	,,
1 in 15,625	12	,,	,,	,,	,,
1 in 78,125	(2·5)	,,	,,	,,	,,

It will be seen that, as in the previous experiment, the rabbit's serum (although it had a definite pink tinge) acquired no increased bactericidal power as a result of the injection of the drug; and that the bile, whether obtained from the normal or the treated animal, possessed no trace of bactericidal power to *B. typhosus*.

SUMMARY.

1. Serum derived from human blood treated with mercurochrome (1 in 40,000 to 1 in 10,000) had no bactericidal power for staphylococcus or hæmolytic streptococcus.

2. Human blood (defibrinated) which had received an addition of 1 in 10,000 of mercurochrome had considerably less power to kill staphylococci than the same blood without mercurochrome.

3. Bile derived from a rabbit which had been given a maximal dose (5 mgm. per kilo) of mercurochrome possessed no bactericidal power for *B. typhosus*. The normal bactericidal power for *B. typhosus* possessed by the serum of this rabbit was not increased by the injection of the drug.

DISCUSSION.

These results suggest that no direct bactericidal effect either in the blood-stream or the bile is likely to follow the administration of mercurochrome. They therefore afford no support to the recommendation that the drug should be employed in septicæmia and for the treatment of typhoid carriers. It may be objected, however, that the clinical records of many cases already published furnish a certain amount of evidence that the use of the drug has in some manner contributed to the recovery of patients suffering from severe septic infections. We do not propose to discuss these clinical records critically, but only to put forward tentatively a possible explanation of them. The records

make it clear that the injection of this drug in man is very frequently followed by formidable constitutional disturbances, as shown by the occurrence of hæmaturia, violent purging, rigors and stomatitis. This being the case, it seems to us possible that the remarkable clinical improvement which is said to have occurred in some of the septicæmic cases within a few hours of the injection of the drug may have been brought about, not by a direct effect upon the microbic infection, but by an auto-immunization process initiated by the severe constitutional disturbance. It is well known that such profound disturbances, for example those manifested by collapse and the occurrence of rigors in "protein shock," may exert a favourable effect upon the course of bacterial infections; and we have evidence (which we are reserving for publication elsewhere) that these favourable effects may sometimes, at any rate, be accompanied by a very striking auto-immunization, as shown by greatly increased bactericidal power of the blood.

The question whether so toxic a drug should deliberately be employed on the off-chance of provoking a profound physiological reaction of this kind we shall leave to others to discuss.

REFERENCES.

Fleming, A.—(1924) *Proc. R. Soc.*, B., **96**, 171.

Hill, Justina H., and Colston, J. A. C.—(1923) *Johns Hopkins Hosp. Bull.*, **34**, 220, 372.

Hill, Justina H., and Scott, W. W.—(1925) *Arch. Int. Med.*, **35**, 503.

Meyer, K. F., Sommer, H., and Eddie, B.—(1926) *J. Infect. Dis.*, **38**, 469.

Walker, J. E.—(1926) *Arch. of Pathol.*, **1**, 199.

Wright, Sir Almroth E., and Colebrook, L.—(1921) 'Technique of the Teat and Capillary Glass Tube,' London (Constable).

Wright, Sir Almroth E., Colebrook, L., and Storer, E. J.—(1923) *Lancet*, **1**, 305, 417, 475.

Young, H. H., Hill, Justina H., and Scott, W. W.—(1925) *Arch. of Surg.*, **10**, 813.

Young, H. H., White, E. C., and Swartz, E. O.—(1919) *J. Amer. Med. Assoc.*, **73**, 1485.

THE TUNGSTEN ARC AS AN EXPERIMENTAL SOURCE OF ULTRA-VIOLET RADIATION.

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THE literature dealing with the effects of ultra-violet radiation, especially in relation to the biological sciences, has grown rapidly within the last decade. This is largely attributable to a wider realization of the properties of these invisible rays, and to the application of them in the laboratory, in technology and in therapeutics. It is unfortunate that the term "ultra-violet *light*" should have gained such widespread usage among even scientific writers. Gradually, however, the term "light" is becoming restricted to that portion in the scale of electro-magnetic waves constituting the visible spectrum.

Most of the work concerning ultra-violet radiation has been done with quartz mercury-vapour lamps, the great advantage of which is the small amount of heat emitted. These tubes are expensive, however, and after a certain time fail to maintain their original output, with a progressive diminution in the amount of radiation obtainable. The tungsten arc, on the contrary, is cheap, and while not as steady from minute to minute as the mercury-vapour tube, remains indefinitely an approximately constant source of radiation. It is also more convenient for work requiring frequent stoppages, and this is specially the case if the mercury-vapour burner be of the atmospheric type. The spectrum of the tungsten arc is more continuous than that of the mercury-vapour lamp. This point is of importance, for it would seem desirable in the future to be able to select a band of radiation of definite wave-length, rather than to apply the heterogeneous emanation which is perforce in use at present. The variety of different wave-lengths available with the tungsten arc would therefore be in excess of that furnished by the mercury-vapour lamp.

Owing to the fact that ultra-violet rays do not penetrate glass, their employment in research has necessitated the use of silica vessels. Recent developments have made possible the production of fused transparent silica in large quantities by means of the electric furnace. The laboratory ware now available is of remarkably good quality, and almost any form of apparatus is procurable. Silica is peculiar in that it can be heated with impunity, and rapid cooling by immersion in water does not result in fracture—a property of great convenience in the sterilization of apparatus.

Control of the Heat Emitted.

A considerable amount of heat is generated while the arc is burning, but apart from the use of a water-jacket, there are three simple methods of overcoming any difficulties arising from this. In this laboratory the interposition of a fused silica plate between the arc and the object to be irradiated has been found exceedingly satisfactory even for exposures up to 20 minutes' duration at 5 in. distance. A simpler method is to irradiate directly for several minutes and allow an interval for cooling. Lastly, the rays may be collected into a parallel beam by a positive quartz lens and directed on to the object at a distance. This is not so satisfactory, however, as heat-rays are also collected by the lens.

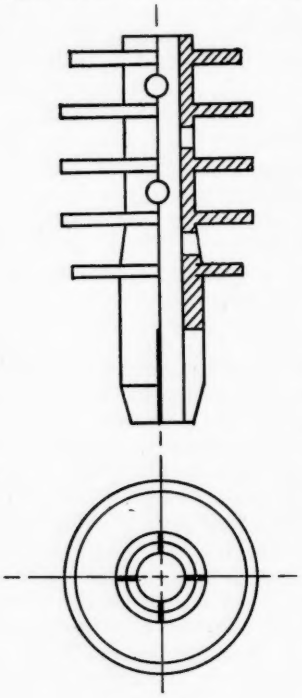

FIG. 1.

An attempt was made to reduce the heat of the arc at its source, and to this end, adopting the principle of air cooling employed on petrol-engine cylinders, the device in Fig. 1 was turned in the laboratory workshop from brass stock. It is a collar which fits on to the tungsten rod a short distance below the tip, and bears several circular vanes. The shank is drilled between the vanes to allow a free circulation of air round the tungsten rod. Sawcuts are made in the lower end and the resulting segments bent inwards slightly, thus forming a push-fit on the rod. This has slightly reduced the heat emitted, and has also diminished the rate of consumption of tungsten.

Apparatus Used.

An ordinary open, hand-feed arc lamp, with mechanical adjustments, has been used in this laboratory. The tungsten and carbon are mounted in the lamp in the usual way, the tungsten being placed below the carbon. The vertical arc thus obtained requires much less attention than when horizontal.

Tungsten rod ($\frac{1}{4}$ in., or less, in diameter) can now be procured easily; it is specially "swaged," or rolled hard, as by this process a steadier arc is ensured. The positive electrode must always be tungsten. The negative electrode may be either an 18 mm. cored carbon, or another tungsten rod. In the latter case there is an increased output of invisible rays, but the addition is relatively small, and in therapeutic lamps a carbon is generally used. Tungsten-cored carbons have been introduced, but the heat generated by them is inconvenient in experimental work. Recently Roberts (1927) has described therapeutic and experimental results observed with an arc employing titanium electrodes. Carbons impregnated with iron and other ores are also in use, and many additional types of electrodes will doubtless be advocated in the future.

It is unnecessary to test for polarity, and no difficulty should be experienced in determining whether connection to the mains has been correctly made. When the tungsten is positive, the arc burns smoothly and with a brilliant bluish-white flame, whereas with the carbon positive there is great spluttering and the light is of an orange tint.

Direct current is essential, and is conveniently taken from the mains. A series resistance, preferably variable, is of course necessary to control the current, and it is desirable to include an ammeter in the circuit. The quantity of radiation emitted seems to vary, within limits, approximately in proportion to the current used.

A decided gain in efficiency is obtained by the use of reflectors. One may be incorporated in the arc lamp to reflect forwards, and another, which is almost essential, may be placed close to the silica vessel under irradiation; if bent to an angle of about 130°, a certain degree of "cross-fire action" results on the distant side of the tube. The best material for this purpose is polished magnalium (an alloy of magnesium and aluminium), but for ordinary work nickel-plate is very suitable.

Points in Technique.

Early experiments to investigate the lethal power of ultra-violet radiation on broth cultures of common micro-organisms emphasized several important points in the technique of irradiation. First, all shadowing must be avoided so that the path of the rays may be unimpeded. Secondly, in the case of fluids which are relatively opaque to the rays, agitation is necessary while irradiation is in progress, so that all parts of the fluid may at some time be brought in contact with the walls of the container. These two points have long been realized, but they are fundamental and will bear repetition. The latter condition is readily fulfilled by bubbling filtered air through the liquid continuously during irradiation. If led by a fine glass capillary tube to the bottom of the silica vessel, the resulting stream of bubbles rising rapidly through the liquid promotes a vigorous circulation. Table I shows the results obtained under identical conditions with and without agitation in this manner:

TABLE I.

Time of exposure (minutes).	No agitation.	Agitation by stream of bubbles—about 120/minute.
0	Solid growth	Solid growth.
½	Slightly less dense	Colonies just confluent.
1	Colonies distinct	Discrete: about 60.
2	Still confluent	15 colonies—all small.
4	Becoming discrete	Sterile.
6	Colonies ,,	,,
8	About 90 colonies	,,
10	,, ,,	,,
12	Smaller colonies—about 80	,,
15	2 c.c. inoculated into bouillon: growth +++	2 c.c. inoculated into bouillon: sterile.

24 hours' *broth* culture of *B. coli*. 7-ampère arc at 5 in. distance: nickel reflector. Loopful inoculated on to agar.

A special form of silica tube, designed to increase the rapidity of action of the rays on a given volume of fluid, is shown in Fig. 2. It is a flattened test-tube, measuring in section ½ in. by ¼ in., and bears a side limb through which a piece of glass tubing drawn out to capillary diameter can be inserted. A short length of rubber tubing makes an air-tight joint between the two. Before use the wool pledget in the glass tube is pushed down into the neck of the capillary, thus forming an air filter, and connection can then be made to a supply of air under slight pressure. The neck of the silica tube is made circular to facilitate plugging with wool. The whole apparatus can be sterilized and stored ready for use.

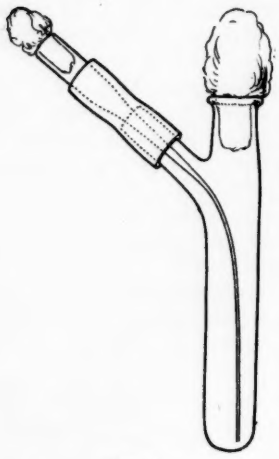

FIG. 2.

In the early tests, incomplete emulsification of the surface growth from agar cultures of organisms was responsible for anomalous results, of which Table II is an example. In this particular case, particles were clearly visible in the liquid.

TABLE II.

Time of exposure (seconds).	Result.
0	Surface covered with small, almost confluent colonies of *B. coli*. *Staph. albus* discrete and about 30 in number.
15	One *Staph. albus* colony. No *B. coli*.
30	Sterile.
45	,,
60	33 discrete colonies *Staph. albus*.
(Minutes.)	
1½, 2, 3, 4	Sterile.
7½, 10	0·5 c.c. inoculated into bouillon: sterile.

B. coli and *Staphylococcus albus* emulsified in *saline*. Loopful inoculated on to agar: 24 hours' culture. ½ in. diameter silica tube; 4 ampères at 4 in. distance. Nickel reflector: little agitation.

Frothing is an annoyance with certain media, and when this occurs the rate of air-bubbling must be reduced and any excess of froth pipetted off at intervals.

It will seem unnecessary to mention that the eyes must be protected by goggles while work is being carried out with these rays, but one experience of the discomfort of the conjunctivitis which carelessness in this respect may lead to is usually sufficient to impress the necessity for caution. Happily the reaction is superficial and transient.

Comparison with a Mercury-Vapour Lamp.

A direct comparison was undertaken between the tungsten arc and a quartz-mercury-vapour burner, the lethal effect on an organism being selected as a test. The burner was of the atmospheric type (Kelvin, Bottomley & Baird), and being relatively new, was presumably yielding its maximum output. It was mounted in a therapeutic stand, which included a reflector and casing of "visor" pattern. The exposures were made by manipulating the casing. Two equal portions of the same saline emulsion of an old culture of *B. subtilis* were exposed to the two sources of rays, conditions being identical in each case. From Table III it will be seen that the tungsten arc proved slightly the more powerful in sterilizing the saline emulsion.

TABLE III.

Time of exposure (seconds).	Mercury-vapour lamp (230 volts).	Tungsten arc: 7 ampères (230 volts).
0	Solid growth	Solid growth.
5	,,	,,
10	,,	Becoming discrete.
15	Becoming discrete	About 85 (colonies).
20	Almost ,,	,, 36 ,,
25	34 (colonies)	,, 19 ,,
30	16 ,,	,, 13 ,,
40	9 ,,	,, 4 ,,
50	15 ,,	,, 1 ,,
60	3 ,,	Sterile.
75	Sterile.	,,
90	,,	,,

Saline emulsion of old culture *B. subtilis*. Nickel reflector: distance 9 in. Vigorous stream of air-bubbles. Loopful inoculated on to agar: 24 hours' culture.

The conditions were rather in favour of the mercury-vapour lamp, for it was run continuously in view of the fact that it is bad practice to start an atmospheric type of burner frequently, and though well shrouded, a number of stray beams escaped and were reflected at a distance from the walls and floor of the room in which the experiment was performed. This no doubt gave rise to a weak continuous irradiative effect in the intervals between exposures. In addition, the casing provided an efficient reflecting surface for the radiation emitted from the back of the burner: it was not possible to reproduce this

with the tungsten arc, which, moreover, was switched off completely at the end of each 5 or 10 seconds' exposure.

As a matter of interest, a similar comparison on the rapidity of production of formaldehyde in a filtered tissue-extract was carried out, using Schryver's (1910) test. In each case a reaction was observed after 5 minutes' irradiation at 9 in. distance.

These results have not been due in any part to ordinary light. Common soda-glass, 2 mm. thick, was found to give complete protection to *B. subtilis* in broth suspension during 20 minutes' exposure, while a tube of the same emulsion irradiated simultaneously behind a silica plate under otherwise identical conditions was sterilized in 4 minutes.

Irradiation has proved extremely useful in these laboratories as a means of dealing with surface contamination of tissue. The lack of penetrative power renders ultra-violet radiation of much greater service in this respect than X-rays. My colleagues have thus repeatedly succeeded in obtaining clean cultures from material received after removal in which absolute asepsis had not been preserved, and in which contamination of cultures had often occurred previously. Indeed, major operations on the human subject have been successfully undertaken with no other means of sterilization than exposure to these rays. It seems also that we have here a method of sterilizing media which would be seriously affected by heating. By suitable means, the exposure necessary could probably be shortened sufficiently to prevent the onset of gross changes in the medium.

Great development and extension of the applications of these rays may be anticipated in the future, and it is possible that in a few years no up-to-date laboratory will be considered complete without its ultra-violet ray installation. Certain it is that in connection with this subject there remains in chemistry, physics and medicine a vast field as yet only imperfectly explored.

These notes conclude with an expression of indebtedness to Prof. Lorrain Smith and to Prof. D. P. D. Wilkie for the laboratory facilities afforded by them to the author. Thanks are also due to Dr. T. Rettie, and to Dr. J. M. Woodburn Morison, for the use of apparatus. My colleague, Mr. C. F. W. Illingworth, has rendered much appreciated assistance.

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REFERENCES.

ROBERTS, A.—(1927) *Brit. Med. J.*, **1**, 184.
SCHRYVER, S. B.—(1910) *Proc. R. Soc.*, **82**, 226.

THE RELATIONSHIPS BETWEEN HEAT-STABLE AGGLUTINOGENS AND SENSITIVITY TO BACTERIOPHAGE IN THE SALMONELLA GROUP.

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SEVERAL observers have reported that bacteria sensitive to a given phage are frequently serologically related. Marcuse (1926) obtained a strongly active phage from a case of dysentery (Type Y infection), and found that it lysed certain laboratory strains of coliform bacilli as well as Y-dysentery bacilli. These coliform strains possessed in common the power to absorb a considerable proportion of the agglutinins from a serum prepared against the original Y-strain. The coliforms insensitive to lysis did not absorb, or absorbed a much smaller proportion of the agglutinins.

Hadley (1926) isolated from sewage by a process of successive filtration and growth with the chosen substrate organism, phages active against *B. pullorum*, *B. gallinarum* and *B. typhosus*. After numerous passages on the homologous organism they were tested for their activity against each of the other two species. All phages were strongly and approximately equally active for each of the organisms. Hadley correlates this with the known serological similarity of these three organisms.

The present communication deals with the same group of organisms as was studied by Hadley, and while confirming his findings, is concerned with a wider range of bacterial species than was used by him.

The serological classification of the Salmonellas that has been used is that published by Bruce-White (1926), and attention has been directed almost solely to the heat-stable antigenic constituents. Weil and Felix (1920) showed that the heat-labile and heat-stable agglutinogenic constituents of bacteria of this group are completely independent, and must be considered separately in examining the serological behaviour of a given strain. The differentiation has been almost universally accepted, and it is generally considered that the H- (heat-labile) agglutinogen represents flagellar or capsular material, while the heat-stable O-factors represent the actual body proteins of the organism.

It will be seen from Bruce-White's table of the serological constitution of the Salmonella group that the O-elements of *enteritidis* have factors in common with a number of other types. The major factor III is common to *enteritidis*, *typhosus* and *pullorum*, while a less important element is shared with *Derby*, *paratyphosus B*, *Stanley*, *Ærtrycke* and *Reading*. (The type names used by Bruce-White will be used throughout the paper.) The characteristic differences between the organisms containing III in common are related to the H-agglutinogens. The fowl typhoid organisms resemble the

dysentery bacilli in possessing no H-element, while the H-factors of *typhosus* and *enteritidis* are quite unrelated.

All the organisms of the group are capable of undergoing "rough" variation, particularly after long existence on laboratory media. The "rough" change may be very variable in its degree, and the type of colony surface or the mode of growth in broth may not be an adequate expression of the amount of serological change that has occurred. In a typical rough culture of *enteritidis*, growth in broth is at first granular and the bacteria rapidly sediment to the bottom; on a MacConkey plate the colonies are finely roughened on the surface and show a very gradually shelving edge. When precautions to prevent spontaneous agglutination are taken, rough strains usually show a floccular (H) agglutination to the approximate titre of a standard *enteritidis* serum. Stable emulsions possessing only heat-stable factors are best obtained by Bruce-White's method of alcohol precipitation and re-emulsification in saline. With such material it has been shown (Arkwright and Goyle, 1924; Bruce-White, 1925) that the change to the R-form is associated with an antigenic change in the heat-stable O-complex of the organism. This is the important element in the serological behaviour of R-strains. Other changes may occur, but the change in the heat-stable complex is probably the best criterion of roughness available.

EXPERIMENTAL.

In studying the behaviour of variants induced by bacteriophage action on strains of *enteritidis*, it was noticed that changes in sensitivity seemed to be related to the nature of the heat-stable agglutinogens. One strain for instance that showed normally smooth colonies and only a moderate sediment in broth behaved toward a series of phages in the way typically rough variants did. Serologically it was found to have a typical "rough" heat-stable complex. This suggested an examination of the two other groups of organisms that have a very similar O-structure to *enteritidis*, and it was immediately evident that the three groups had almost identical phage sensitivities. The phage samples were derived from a variety of sources—rat fæces, fowl fæces, lysogenic strains of *enteritidis*, etc.* They were grown for several generations on the *enteritidis* strain chosen as substrate, and each was derived by successive isolation from a single plaque on agar. Isolation was continued until the plaques showed a reasonable uniformity in their diameter and general appearance. When this occurred one further plating was made and the stock phage derived from a single plaque, subcultures being made occasionally as required. No effort was made to secure a maximum virulence, and the cultures were usually sterilized (as regards bacteria) by heating to 57° for 30 minutes.

The series of phages used in these experiments was selected from a considerably larger number, after numerous experiments (according to the methods of Bail (1923) and his school) had shown that each possessed different

* Phage 8 was obtained from rat fæces; No. 11 received from Dr. I. N. Asheshov, source unknown. Nos. 1, 12 and 13 were obtained from the interaction of phage 11 with a lysogenic strain of *enteritidis*; No 18 was developed from a Shiga phage found in fowl fæces; Nos. 20, 21 and 22 were obtained from lysogenic *enteritidis* cultures.

individual characteristics, *i. e.* no two produced resistant strains that showed the same qualitative range of sensitivity toward the phages tested. Nos. 11 and 13 were large plaque-producing phages; 1, 18 and 21 produced medium-sized plaques; 8, 12, 20 and 22 small ones. Similarities were evident between 11 and 13 and between 1 and 21, but the others seemed functionally quite distinct.

The experimental method found most convenient and adequate for the purpose was as follows: The strain to be examined was grown in broth, and a few drops of the young culture spread over the surface of a 1·2% nutrient agar plate. The plate was dried off in the incubator and small areas were spread with a loopful of undiluted phage. On incubation these areas were unaffected in the case of resistant forms, or showed lysis with more or less secondary growth if the organisms were sensitive. The method of course does not show small differences of resistance, and it is certain that lysis under such conditions is not incompatible with a fairly high degree of resistance. Thus it was found that presumably resistant cultures derived from the action of phage 13 on a rough *enteritidis* strain when tested in this way showed lysis by the same phage, but the plaques were only few in number even with undiluted phage. A high degree of partial resistance was therefore present. However, a good deal of evidence, *e. g.* that brought forward by Schurmann (1925), goes to show that complete resistance is more definite an entity than partial resistance. That is to say that for a given "pure" phage there is a range of organisms that it cannot attack. There is another range over which it shows some activity, slight or great, but in all cases capable of being developed to a fairly high level.

Clear-cut positive or negative results were nearly always obtained, but occasionally with the more distantly related strains particularly, a very heavy secondary growth might mask a primary lysis. In a few cases only a few discrete plaques appeared with undiluted phage. The results are shown in tabular form, the degree of lysis being indicated by the number of +'s. The numbers of the bacterial strains refer to those obtained from the National Collection of Type Cultures. In all the tables the results are grouped under three types, α, β, γ, to show the similarity between the species, and any exceptions are shown separately.

TABLE I.—*B. enteritidis.*

	α. Normal smooth type: Wa. 75, 125, 128, 205, 206, 305, 352, 617, 618, 910, 2109, 2212.	β. Rough forms: B.T.M. 127A, 203, 410.	Mixed types: 2202, 2203.	γ. Smooth types: 126, 780, 2187, 2204.
Phage 1	+++	−	+++	−
„ 8	+++	+++	+++	+++
„ 11	−	+++	+	−
„ 12	+++	−	+++	−
„ 13	−	+++	+	−
„ 18	−	+++	+	−

The mixed type probably represent merely an admixture of a proportion of rough

bacteria in the growth. The appearance denoted + was a translucency without real clearing. As the table contains all the strains available in the National Collection, the commonly occurring forms evidently fall regularly into the three groups shown as regards their behaviour to phage. By growth with one or more phages *enteritidis* strains with quite different phage sensitivities can be developed, but apparently the three groups shown above are the only permanently stable ones.

TABLE II.—*B. typhosus.*

	α. Smooth type: 1625, 1777, 2137.	β. Rough forms: 159, 160, 786.	γ. Smooth: 163, 171, 172, 779, 2128, 2132, 2133.	Variants.		
				153.	1598.	901·0.
Phage 1	+++	−	−	+++	−	+
,, 8	+++	+++	+++	−	+++	+++
,, 11	−	+++	−	−	−	−
,, 12	+++	−	−	+++	+++	−
,, 13	−	+++	−	−	+++	−
,, 18	−	+++	−	−	+++	+++

The bulk of the strains fall into the same three types, although in this case there are several variants showing irregular sensitivities, and a larger portion fall into the unsatisfactory γ group in being sensitive only to phage 8.

TABLE III.—*B. pullorum.*

	α. Smooth type: 396.	β. Rough (Jeffersoni) type: 1500, 396A.
Phage 1	+++	−
,, 8	+++	+++
,, 11	−	+++
,, 12	+++	−
,, 13	−	+++
,, 18	−	+++

Only a limited number of cultures were available, but the smooth and rough strains available showed the typical phage sensitivity of the corresponding types of *enteritidis* and *typhosus*.

Serological Data for the Strains Used.

Sera were made from *enteritidis* strains Wa., a typical form used by Bruce-White, and B.T.M., a form giving smooth colonies, but developing a sediment in broth, and showing a sensitivity to bacteriophage characteristic of "rough" forms.

Both sera had H-agglutination titres for the homologous organisms of 25,600. Practically all the work was done with O-emulsions prepared by washing off an agar growth with a small amount of distilled water, adding three or four times the volume of absolute alcohol and allowing to stand at 37° for an hour before being centrifuged. The sediment emulsified in normal saline to an opacity approximately corresponding to 500 million per c.c. gave the emulsion used for agglutination.

The following table shows the general correspondence of both S and R agglutinogens through the three bacterial species. The figure opposite the strain gives the titre of good, not complete agglutination:

TABLE IV.

Strain.	Type of phage sensitivity R or S.	Wa. serum (S).	B.T.M. serum (R).
Enteritidis Wa.	Sα	1280	320
,, B.T.M.	Rβ	160	2560
,, 780	Sγ	2560	1280
Typhosus 1777	Sα	6000	1500
,, 2137	Sα	6000	640
,, 159	Rβ	1280	2000
,, 786	Rβ	6000	2560
,, 2128	Sγ	2800	320
,, 2133	Sγ	1280	200
Pullorum 396	Sα	5120	160
,, 1500	Rβ	80	5120

The two sera give sharp differentiation between smooth and rough *enteritidis* or *pullorum* strains, but there is less differentiation between the typhoid forms. This lack of sharp antigenic distinction between S and R may be related to the greater tendency to exhibit atypical phage sensitivities shown by *typhosus*.

The results may be summarized as showing that the phage reactions of the three bacterial species considered are practically identical. The strains found in stock cultures fall into three groups as far as their sensitivity to phage is concerned in the case of *enteritidis* and *typhosus*, while *pullorum* may lack one of these. If the two groups S α and R β common to the three species are considered alone, it will be seen that their behaviour toward phage seems to depend only upon the nature of their heat-stable agglutinogens. It becomes probable, therefore, that these agglutinogens are intimately related to the specific adsorption of the lytic principle which is the first stage in the process of lysis by bacteriophage.

Some other evidence bearing on this point may be obtained from studying the variants produced in *enteritidis* strains under the influence of various phages. Using the antigenically "rough" strain B.T.M. previously described, a resistant form, B.T.M./13, derived from a culture lysed by phage 13 was obtained. This strain was consistently "smooth" in its behaviour, showed a normal 0-complex serologically and was more virulent to mice than the original. Its sensitivity towards phage was that of a normal *enteritidis* except that it was insensitive to phage 1. This was found to be due to the fact that B.T.M/13, like the parent strain B.T.M., was spontaneously lysogenic, giving a very weak type 1 phage. In this case the change of the heat-stable complex from "R" to "S" is accompanied by a corresponding change in phage sensitivity.

The converse change from "S" to "R" is much more readily obtainable, and is always associated with development of typical "R" sensitivity to phage. Two examples having some other points of interest may be given. Strain Wa after growth in broth with phage 12 for five days gave two types of resistant colonies on plating, both typically rough, but one much more opaque than the other. Serologically the following results were obtained:

Strain.	Serum B.T.M.	Serum Wa.
Wa/12 T.R.	H < 400	O 160
	O 1280	—
Wa/12 O.R.	H 51200	—
	O 5120	O 640

i.e. both forms possess a "rough" heat-stable complex, but the transparent one (Wa/12 T.R.) shows no H-agglutinogen that can be demonstrated by simple agglutination, while the opaque type (Wa/12 O. R.) possesses it in normal amount. Despite this difference, both were sensitive to the usual range of phages, *i.e.* 8, 11, 13 and 18.

A rough form can be obtained from *enteritidis 780*, which is a smooth γ type, by the action of phage 22. This variant is sensitive to phages 8, 13, 18 and 20, so that with the exception of a resistance to 11 its behaviour is the same as that of rough derivatives of *a enteritidis* types.

The three species so far studied have all possessed in common what Bruce-White considers the major O-constituent III, but there are a number of other Salmonellas that possess heat-stable agglutinogenic elements in common with *enteritidis*. These are the *paratyphosus B*, *Ærtrycke* (mutton), *Derby*, *Reading*, *Stanley* and *abortus equi* types. It was impracticable to make a comprehensive examination of the Salmonella forms, but most of the type strains in both "S" and "R" state have been tested for their phage sensitivity. Three further phages, 20, 21, 22, not used in the earlier part of this work are also added. Representative types of each species only were tested against these three phages. Table V summarizes the main features of the Salmonella group in its relation to the O-agglutinins and phage types developed by *enteritidis*.

SUMMARY AND DISCUSSION.

It is generally agreed that the first stage in transmissible lysis is a specific adsorption of the lytic particles to the sensitive bacteria. An active adsorption is most easily demonstrated with a highly active Shiga phage. With less active phages it takes place more slowly, and may be masked by the commencement of multiplication before a considerable proportion of the corpuscles are bound. By the use of low temperatures to inhibit bacterial multiplication, however, the characteristic binding can be demonstrated (Lepper, 1923). Opinion is divided as to the degree of specificity of this binding. Prausnitz and others consider that it is strictly related to lytic power, only bacteria that are sensitive to lysis binding a given phage. A number of well-authenticated exceptions have been described, however, and one can probably state that while adsorption is most rapid and complete to the sensitive homologous organism, a certain amount occurs to related non-sensitive organisms, including some at least of the resistant forms induced by the action of the given phage.

On the whole it is probable that one of the chief factors determining whether or not a bacterial strain will be lysed by a given phage is the intensity with which it adsorbs the phage.

The present paper is concerned with the possible nature of the bacterial constituents responsible for such specific adsorption. Meissner (1925) has

TABLE V.—*Summary of Salmonella Sensitivities to Phages Developed with* Enteritidis *Strains.*

Organism.	Heat-stable agglutinogenic constituents in common with *enteritidis* (Bruce-White).	Sensitivity of "smooth" form to phage.									Sensitivity of "rough" form to phage.								
		1	8	11	12	13	18	20	21	22	1	8	11	12	13	18	20	21	22
B. enteriditis α	III—8	+	+	−	+	−	−	+	+	−	−	+	+	−	+	+	+	−	−
„ γ		−	+	−	−	−	−	−	−	+	−	+	−	−	+	+	+	−	−
B. pullorum	III	+	+	−	+	−	−	+	+	−	−	+	+	−	+	+	+	−	−
B. typhosus α	III - 8	+	+	−	+	−	−	+	+	−	−	+	+	—	+	+	...	−	−
„ γ		−	+	−	−	−	−	−	−	−	...	...	...	...	...	...	...	...	...
B. paratyphosus B	8	−	+	−	+	−	−	+	±	±	−	+	+	−	+	+	±	−	−
Salmonella types:																			
Stanley	8	(±)	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−
Ærtrycke	8	...	...	...	...	...	...	...	...	...	−	+	+	−	+	−	−	−	−
Derby	8	+	−	−	(±)	−	−	−	+	−	...	...	...	...	...	...	...	...	...
Reading	8	(±)	(±)	−	−	−	−	−	−	−	−	+	+	−	+	±	−	−	−
B. paratyphosus A	*Nil*	−	−	−	−	−	−	+	−	−	−	+	+	−	+	±	+	−	−
Newport	„	−	−	−	−	−	−	−	−	−	−	−	±	−	±	−	−	−	−
Suipestifer (Hirschfeld)	„	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−
„ (Arkansas)	„	...	...	...	...	...	...	...	...	...	−	+	+	−	+	+	+	−	−
L.	„	−	−	−	−	−	−	−	−	−	−	+	+	−	+	±	−	−	−

+, active lysis. ±, weak lysis, either a few plaques only, or clearing with heavy secondary growth. ( ) signifies that result shown was not obtained in all tests with the organism.

shown that the power to bind phage is not confined to the living organism, but persists in the heat-killed bacteria, and is only destroyed by a temperature above 110°. Some heat-stable surface constituent is therefore responsible, and the experimental evidence given above makes it extremely likely that the constituent is the same as (or at least in part identical with) that responsible for the phenomena that are ascribed to the functioning of the heat-stable or O-agglutinogens of Weil and Felix.

It has been shown that amongst the Salmonella types those possessing the same heat-stable agglutinogens as *enteritidis* react similarly to a series of phages that have been developed on *enteritidis* strains.

With a few exceptions that do not seem to invalidate the general co-ordination, *enteritidis*, *pullorum* and *typhosus* strains fall into three groups as regards their sensitivity to the phages used. One of these groups (β) includes all the rough forms, and an occasional strain that lacks some of the "rough" characteristics but is antigenically of the group. Of the two "smooth" groups, (α) and (γ), the latter was not found in the case of *pullorum*, but both are evident amongst *enteritidis* and *typhosus* strains. These three species can then be considered from the point of view of their sensitivity to phage as a compact group, and it is reasonable to relate this to the other common factor present, the O-agglutinogen.

The organisms which have a minor degree of similarity to *enteritidis* in their O-structure, *paratyphosus B*, etc., show more or less sensitivity to one or more of the phages used, but with a partial exception in the case of *paratyphosus B* there is no resemblance to the typical behaviour of the more closely related forms. The strains with no O-agglutination in common show no sensitivity whatever to *enteritidis* phages, again with one exception. The "R" forms of all the Salmonellas, however, show much the same behaviour toward phage—a fact that can be correlated with their "serological cosmopolitanism" (Schutze).

This co-ordination between agglutinin-absorbing capacity of an organism and what is probably its specific phage-adsorbing capacity may have some importance for the general theory of bacteriophage, particularly with regard to questions of its variability and power of adaptation. On the whole it seems to point strongly toward the biological independence of the phage particles, but the evidence needs considerable extension before any general conclusions can be drawn.

CONCLUSIONS.

1. From a consideration of their sensitivity to a series of bacteriophage types the three Salmonella species *B. enteritidis*, *B. typhosus* and *B. pullorum*, which have practically identical heat-stable agglutinogens, form a homogeneous group.

2. According to the strain chosen one of three types of reaction toward phage may be shown, each type being common to the three species, except that in the case of *pullorum* one type has not yet been demonstrated.

3. Amongst Salmonella types outside this group, the degree of relationship to *enteritidis* as judged. (1) by the presence of heat-stable agglutinogens in

common and (2) by the sensitivity to phages developed on *enteritidis*, shows an approximate correspondence.

4. Phages developed on "rough" *enteritidis* cultures are active against "rough" variants of most of the Salmonella types, this wide range corresponding to the parallel extent of common heat-stable agglutinogens amongst such forms.

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REFERENCES.

ARKWRIGHT, J. A., AND GOYLE, A. N.—(1924) *Brit. J. Exper. Pathol.*, **5**, 104.
BAIL, O.—(1923) *Zeitschr. f. Immunitätsf.*, etc., **38**, 57.
BRUCE-WHITE, P.—(1925, 1926) *Med. Res. Council Special Reports*, Nos. 94 and 103. London (H.M. Stationery Office).
HADLEY, P.—(1926) *Proc. Soc. Exp. Biol. & Med.*, **23**, 443.
LEPPER, E. H.—(1923) *Brit. J. Exper. Pathol.*, **4**, 53.
MARCUSE, K.—(1926) *Zeitschr. f. Hyg.*, **105**, 17.
MEISSNER, G.—(1925) *Centr. f. Bakt.*, I, Orig. **93**, 489.
WEIL, E., AND FELIX, A.—(1920) *Zeitschr. f. Immunitätsf.*, **29**, 24.

EXPERIMENTAL SENSITIZATION TO CULTURE-FILTRATES OF *STREPTOCOCCUS SCARLATINÆ.*

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THOUGH the Dick test for susceptibility to scarlatina has appeared to be analogous in some respects to the Schick reaction, it is now well recognized that the two phenomena show certain striking differences. The active principle or "toxin" in culture-filtrates (or other preparations) of *Streptococcus scarlatinæ* used for eliciting the Dick reaction exhibits a considerable degree of thermostability, and may only be inactivated by exposure at 100° C. for one hour. In this respect it is unlike diphtheria toxin and certain other exotoxins. Attention has also been drawn to the fact that the positive Dick reaction shows some correspondence to a cutaneous reaction dependent on hypersensitiveness to protein substances, as exemplified by the pseudo-reactions seen in testing for the Schick phenomenon. The Dick reaction, in fact, shows an obvious dissimilarity to the Schick reaction, both in the features of the cutaneous lesion and in the time of its appearance and duration. It is now well known

also that animals are generally insusceptible to culture-filtrates of *Streptococcus scarlatinæ*, even when amounts corresponding to many thousands of "skin-test" doses (for a positively-reacting person) are injected, though, according to Kirkbride and Wheeler (1926), young goats are definitely susceptible to intracutaneous injections. The suggestion has also been put forward that the scarlatinal rash might be interpreted as an allergic phenomenon resulting from hypersensitiveness to the specific proteins of certain hæmolytic streptococci, this allergic state being brought about by previous "subinfection" with these organisms (see Bristol, 1923). On this basis the Dick reaction might be regarded as an index of such sensitiveness, and the negative reaction characteristic of convalescent scarlatina cases might be the result of desensitization during the illness.

Though, normally, animals are usually quite insusceptible to filtrates of *Streptococcus scarlatinæ* cultures, various workers have been able to render laboratory animals sensitive. Paraf (1925) found that rabbits could be sensitized. Dochez and Sherman (1925) have stated that guinea-pigs sensitized by subcutaneous injections of dead cultures, living cultures or culture filtrates of *Streptococcus scarlatinæ* exhibited a cutaneous reaction after intradermal injection of the filtrate, apparently corresponding to the positive Dick reaction in the human subject. They found the reaction was at a maximum a month after the last sensitizing dose. This reaction could be annulled by the injection of an "antitoxic" serum along with the filtrate, the serum being mixed with the filtrate and incubated for one hour before injection. In their published communication the dosage employed for sensitization and the concentration of filtrate used for eliciting the skin-tests are not stated, and no reference is made to control tests with heated filtrate as in routine testing for susceptibility to scarlatina. Zinsser and Grinnell (1925) have found that the best method of sensitizing guinea-pigs is by intraperitoneal injection of living organisms. Sensitiveness developed within a month and was always considerable in degree. For demonstrating cutaneous allergy by intradermal injection, the filtrate of a culture was found to be the most effective preparation. Animals remained sensitive for about six weeks after the last injection. Control tests with heated filtrate are not referred to.

With a view to studying further the experimental sensitization of animals to hæmolytic streptococci of scarlatinal origin, and in order to ascertain whether the cutaneous reaction in sensitized animals following the intradermal injection of culture-filtrates is analogous to the Dick reaction, guinea-pigs have been sensitized and tested in accordance with the method of Zinsser and Grinnell (*v. supra*).

Strains of hæmolytic streptococci isolated from cases of scarlatina were used which were known to produce a filterable principle capable of evoking the Dick reaction in susceptible persons when injected intracutaneously in high dilutions (*e. g.* 1 in 1000). The animals were injected intraperitoneally with living organisms. Young cultures in a veal-extract phosphate bouillon were employed, the organisms being separated by centrifuging and then suspended in saline solution. The suspensions were standardized by opacity standards so that the dosage could be regulated. The injections were repeated at intervals of two to three days and the number of injections and the total dose were varied in different sets of animals. Sensitiveness was tested for by means of filtrates of five days' blood bouillon cultures of the particular strain used; the blood added to the medium was obtained from guinea-pigs—the same species as the animals tested. 0·2 c.c. of filtrate diluted in saline (*v. infra*) was injected

intradermally with a fine needle into an area of skin over the abdomen, shaved a day or two before the injections. Before commencing treatment, corresponding cutaneous tests were carried out to exclude any natural sensitiveness to these filtrates. No untreated animal ever showed a positive reaction.

Forty-eight animals were treated in this way, and apart from controls, over two hundred cutaneous tests with filtrates of *Streptococcus scarlatinæ* and other streptococci were carried out. It was found possible, under certain conditions, to sensitize guinea-pigs so that the intradermal injection of a culture-filtrate produced a cutaneous reaction. Generally, however, only high concentrations of the filtrate were effective, *e. g.* 1 in 5 or 1 in 10. Weaker dilutions, *e. g.* 1 in 50, with few exceptions, yielded no reaction. Sensitization was variable. Different animals varied in the degree of the cutaneous reactions which they showed and some animals remained non-sensitive.

The total dose of organisms varied in different sets of animals from 1900 to 40,000 millions approximately; the number of injections varied from 7 to 18. The most effective system was found to be a series of 7 to 9 graded doses, the total amount varying from 8000 to 22,000 million organisms, with an initial dose of 60 to 180 millions. Larger doses produced less satisfactory results. Cutaneous tests were carried out after 1 to 4 days from the completion of the injections and sensitiveness was usually noted on first testing. On repeated testing, the sensitiveness was found to persist up to the 40th day, though diminishing in degree after 7 to 10 days. In some cases there was only a transitory sensitiveness, which disappeared after a week or a fortnight. In an additional set of animals an attempt was made to sensitize by injections of filtrate alone, but without result on repeated testing up to the 21st day from the last injection. In one set of animals, filtrate was injected along with living organisms without any apparent intensification of the sensitive state as compared with guinea-pigs that received the same doses of living organisms alone.

The reaction.—The cutaneous reaction varied considerably; in certain instances there was a well-delimited erythematous area with swelling and infiltration of the cutis; in others, the erythema was less pronounced, less defined and unaccompanied by obvious swelling and infiltration. The size of these areas varied from 5 to 15 mm. in diameter. The reaction developed usually after 9 to 12 hours and began to fade after 18 hours. The more marked reactions undoubtedly corresponded to a Dick reaction—of moderate degree—both in their general appearance and in the time of development and fading. In some cases only a small red infiltrated papule (4–5 mm. in diameter) resulted without any diffuse erythema. This was the minimum effect recorded as positive, but a reaction even of the slightest degree never occurred in normal animals.

Of the 48 animals treated with living organisms, the reactions elicited shortly after completion of treatment were classified as follows (the number of + marks denoting the relative degrees of the various reactions elicited): +++, 5; ++, 13; +, 14; *nil*, 16; total positive reactions, 32. Thus, only in a minority of the treated animals was the cutaneous sensitiveness of marked degree.

In 22 of the 32 positively reacting animals control tests were carried out,

in which filtrate heated at 100° C. for 1½ hours was injected at the same time as the unheated material and in equal amount. Sherwood (1926) has shown that when high concentrations of filtrate are used in the Dick test, the control material must be heated at 100° C. for 1½ hours to ensure complete inactivation. In the control tests the reaction was identical with that produced by unheated filtrate in 13 animals, less in degree in 6, and absent in 3. In the majority of cases, therefore, the reaction to the heated filtrate was identical with that in the actual test.

Tables I and II serve to illustrate comparative reactions with unheated filtrate and filtrate heated at 100° C. for 1½ hours. Table I also shows the decreased sensitiveness after 10 days. In further tests in which control tests were carried out with filtrate heated for even 3½ hours at 100° C., the active principle appeared to remain stable (Table III).

TABLE I.—*Guinea-pig Sensitized with* S. scarlatinæ.

Total dose 8000 million living organisms; 8 doses graded from 60 to 1800 millions; filtrate diluted 1 in 10 for intradermal tests.

Day after last sensitizing injection.	Cutaneous reactions.	
	Unheated filtrate.	Heated filtrate.
6th	+++	++
10th	+++	++
13th	++	++
27th	+	+
35th	+	+

TABLE II.—*Guinea-pigs Sensitized with* S. scarlatinæ.

Total dose 16,000 million living organisms; 9 doses graded from 180 to 4800 millions; filtrate diluted 1 in 10 for intradermal tests.

Animal.	Day after last sensitizing injection.	Cutaneous reactions.	
		Unheated filtrate.	Heated filtrate.
1	1st	++	++
2	,,	+	+
3	,,	+	+
4	,,	+++	++
5	,,	++	++

TABLE III.—*Guinea-pigs Sensitized with* S. scarlatinæ.

Total dose 8100 million living organisms; 7 doses graded from 180 to 3600 millions; filtrate diluted 1 in 5 for intradermal tests.

Animal Set VIII.	Day after last sensitizing injection.	Cutaneous reactions on intradermal injection of filtrate.			
		Unheated.	1½ hrs. 100° C.	2 hrs. 100° C.	3½ hrs. 100° C.
1	29th	+	++	+	+
2	,,	++	++	++	++
3	,,	+	+	+	+

In order to ascertain the degree of specificity of the reaction, parallel tests were made with filtrates from other hæmolytic streptococci, including scarlatina strains of the same serological type as that used for sensitization, strains of different serological type and non-scarlatina strains isolated from simple suppurative lesions. These last included organisms which yielded a filtrate capable of eliciting the Dick reaction if injected in sufficiently high concentration (see McLachlan, 1927), and also strains which were inactive in this respect. In general, animals that reacted to the filtrate of the strain used for sensitization showed reactions also with filtrates of the other streptococci, in most cases to an equal degree, though in some instances weaker reactions were obtained with the heterologous filtrates (Table IV).

TABLE IV.—*Guinea-pigs Sensitized with* S. scarlatinæ.

Total dose 16,000 million living organisms; 9 doses graded from 180 to 4800 millions filtrate diluted 1 in 10 for intradermal tests.

Animal Set II.	Day after last sensitizing injection.	Cutaneous reaction with filtrates.				
		Scarlatina streptococci.			Non-scarlatina streptococci (hæmolytic).	
		Strain used for sensitization.	Strain "2."	Strain "36."	Strain "D."	Strain "A."
1	7th	+	+	+	+	...
	30th	+	...	...	...	+
2	7th	++	++	++	+++	...
	30th	−	...	...	...	−
3	7th	+++	++	++	+	...
	30th	−	...	...	...	−
4	7th	+++	++	++	+	...
	30th	+	...	...	...	+
5	7th	++	++	++	...	++
	30th	−	−	−	−	...
6	7th	+	+	++	++	...
	30th	+	...	...	...	+

Strain "2," of same serological type as that used for sensitization.
„ "36," of different serological type.
„ "D," filtrate active in Dick test when used in high concentration (1 in 100).
„ "A," filtrate quite inactive in Dick test even in high concentration.
In a series of normal control animals tested with filtrates (1 in 5) of these strains no positive reactions occurred. The treated animals had been tested before sensitization, with negative result, as in all the experiments.

Thus no definite specificity could be claimed for the reaction, and in further tests non-specific cutaneous reactions were noted with similar filtrates from a non-hæmolytic streptococcus, a strain of pneumococcus type I and *Staphylococcus aureus* (Table V).

TABLE V.—*Guinea-pigs Sensitized with* S. scarlatinæ.

Total dose 27,000 million living organisms; 9 doses graded from 600 to 6000 millions; filtrates diluted 1 in 10 for intradermal tests.

Animal Set V.	Day after last sensitizing injection.	Cutaneous reaction with filtrates.			
		Strain used for sensitization.	Non-hæmolytic streptococcus.	Pneumococcus Type I.	*Staphylococcus aureus.*
1	15th	—	—	—	—
2	,,	++	+	+	++
3	,,	++	++	+	+
4	,,	+	—	+	+
5	,,	—	—	—	—
6	,,	—	—	—	—

In a series of normal control animals tested with filtrates (1 in 5) of these organisms no positive reactions were noted, except in one animal out of eight tested with a filtrate of the non-hæmolytic streptococcus; in this animal a slight reaction approximating to that classified as + was noted. The treated animals had been tested before sensitization, with negative result, as in all the experiments.

Observations were made in a series of sensitive animals to ascertain whether the active principle of the filtrate was neutralized by a scarlatina antitoxic serum as claimed by Dochez and Sherman (1925). A Parke-Davis serum was used. The serum was diluted 1 in 25 and mixed with a 1 in 5 dilution of filtrate. 0·2 c.c. of the mixture was injected intradermally. These relative amounts were estimated from the amount of serum required to neutralize a 1 in 500 dilution of the filtrate when injected intradermally in susceptible persons. No neutralization effects were noted in the sensitized guinea-pigs, and, in fact, these animals showed a certain degree of cutaneous sensitiveness to the serum by itself, so that the injection of filtrate *plus* serum yielded a more marked reaction than the filtrate alone.

CONCLUSIONS.

Though it has been possible, under certain conditions, to produce cutaneous sensitiveness in guinea-pigs to culture-filtrates of scarlatina streptococci by injection of living organism, the cutaneous reactions are not definitely comparable with the Dick reaction in persons susceptible to scarlatina. These reactions are elicited only by the intradermal injection of high concentrations of filtrates and are rarely of marked degree. Generally the active principle in filtrates is not inactivated at 100° C., even after 3½ hours' exposure, and the sensitive state is not specific.

REFERENCES.

BRISTOL, L. D.—(1923) *Amer. J. Med. Sci.*, **166**, 853.
DOCHEZ, A. R., AND SHERMAN, L.—(1925) *Proc. Soc. Exper. Biol. and Med.*, **22**, 282.
KIRKBRIDE, M. B., AND WHEELER, M. W.—(1926) *J. Immunol.*, **11**, 477.
McLACHLAN, D. G. S.—(1927) *J. Hyg.*, **26**, 84.
PARAF, J.—(1925) *Bull. et Mém. Soc. méd. d. Hôp. de Paris*, **49**, 395.
SHERWOOD, N. P.—(1926) *J. Immunol.*, **11**, 331.
SMITH, J.—(1926) *J. Hyg.*, **25**, 165.
ZINSSER, H., AND GRINNELL, F. B.—(1925) *J. Immunol.*, **10**, 725.

THE INFLUENCE OF COAGULATION ON THE BACTERICIDAL POWER OF THE BLOOD.

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FOLLOWING our suggestion (1926) that the superiority of defibrinated blood over citrated blood in respect to the bactericidal power was due to the fact that defibrinated blood is virtually clotted, these experiments were carried out as a further investigation of the influence of clotting on the bactericidal power of the blood.

In the first series of these experiments, blood, which after infection had clotted in the slide-cell, was compared with citrated and with defibrinated blood.

First Series of Experiments.

Blood was taken from the vein of a normal subject and 100 c.mm. of the blood mixed with 2·5 c.mm. of a suitable dilution of a 24 hours old broth culture of staphylococcus. The infected sample of blood was then transferred to a slide-cell, clotting taking place in the cell.

To another 100 c.mm. of blood $\frac{1}{20}$ of its bulk of 5% sodium citrate was added, which prevented clotting. 2·5 c.mm. of the same dilution of staphylococcus was added to the citrated sample, which was then transferred to a slide-cell. The cells were incubated at 37° centigrade, and after 24 hours the number of colonies which had grown in each cell counted. This was repeated six times, as follows:

	Number of colonies growing in cell.					
	Exp. 1.	Exp. 2.	Exp. 3.	Exp. 4.	Exp. 5.	Exp. 6.
Citrated blood . .	106	155	140	160	25	12
Clotted blood . .	51	12	7	19	0	0

In the following experiment defibrinated blood was compared with blood which had clotted in the cell. The same procedure as before was adopted:

	Number of colonies growing in cell.			
	Exp. 1.	Exp. 2.	Exp. 3.	Exp. 4.
Defibrinated blood	32	12	16	10
Clotted blood	10	6	10	2

In the foregoing experiments the blood which had clotted in the cell was superior to the citrated blood. The difference between citrated and clotted blood was much greater than between defibrinated blood and clotted blood, which is in accordance with the view that clotting increases the bactericidal power, as defibrinated blood has gone through the stages of coagulation.

Second Series of Experiments.

In this series of experiments, blood which had been prevented from clotting by the addition of hirudin was compared with citrated blood. Blood was taken as before and divided into two equal parts. Both parts were prevented from clotting, in the one case by the addition of $\frac{1}{20}$ of its bulk of 5% sodium citrate, and in the other case by the addition of $\frac{1}{20}$ of its bulk of a suitable dilution of hirudin.

An equal amount of hirudin was also added to a sample of citrated blood. 100 c.mm. of each sample was now infected with 2·5 c.mm. of the same dilution of staphylococcus and transferred to the slide-cells. After 24 hours' incubation the number of colonies growing in the cells were counted:

	Number of colonies growing in cell.	
	Exp. 1.	Exp. 2.
Citrated blood	408	348
Hirudinized blood	72	46
Citrated blood plus hirudin	310	307
Citrated blood	295	301
Hirudinized blood	46	66

As will be seen from the above experiments, the superiority of hirudinized blood over citrated blood could not be accounted for by any bactericidal action of hirudin itself. In the case of citrated blood, however, clotting had been prevented in the early stages, whilst in the case of hirudinized blood all the stages of clotting had been gone through up to the formation of thrombin, and only the last stage being prevented by the destructive action of hirudin on thrombin as it was formed.

Third Series of Experiments.

In this series of experiments blood which was citrated at increasing intervals of time was compared.

Blood was taken from a vein as before and placed in a sterile tube on ice. 300 c.mm. of the blood was immediately removed from the tube and $\frac{1}{10}$ of its bulk of 5% sodium citrate added to it. After an interval of 4 minutes another 300 c.mm. of blood was removed from the tube and citrated. This was repeated at the end of 6, 8 and 10 minutes.

We now had five samples of citrated blood, one sample citrated immediately on shedding, the other four samples citrated at intervals of 4, 6, 8 and 10 minutes after shedding. 75 c.mm. of each sample was infected with an equal amount of the same dilution of staphylococcus, transferred to the slide-cells, incubated as before, and the number of colonies which had grown counted:

	Number of colonies growing in cell.	
	Exp. 1.	Exp. 2.
Blood citrated immediately after shedding	64	57
„ 4 minutes after shedding	44	27
„ 6 „ „	36	41
„ 8 „ „	33	19
„ 10 „ „	18	12

In the above experiments the blood in the cooled tube clotted in 12 minutes. In the following experiments the blood was cooled to a greater extent, clotting in the tube taking place in 23 minutes.

	Number of colonies growing in cell.	
	Exp. 1.	Exp. 2.
Blood citrated immediately after shedding	66	68
„ 3 minutes after shedding	53	46
„ 6 „ „	28	26
„ 9 „ „	26	25
„ 12 „ „	41	23
„ 15 „ „	26	25
„ 18 „ „	22	10
„ 21 „ „	20	10

It would appear from these experiments that as blood clots it develops bactericidal properties.

Various workers have made it clear that hirudin contains a substance destructive to thrombin. Whether it also possesses the power of destroying thrombokinase is extremely difficult to determine, for the only clear evidence for the presence of thrombokinase is its power of producing thrombin. The fact that hirudin prevents thrombokinase from showing its thrombin-producing activities is therefore no proof that hirudin destroys thrombokinase. Although we cannot exclude the possibility of hirudin containing an anti-thrombokinase, it is legitimate to adopt the hypothesis that hirudin prevents coagulation by destroying thrombin, whilst permitting the stages of clotting to proceed as far as the formation of thrombin.

Such an action is in marked contrast to the action of citrate, which prevents the formation of thrombin by removing one of the precursors of thrombin, namely, calcium, from the field of activity. Whether this view of the action of hirudin be justified or no, the foregoing experiments have at least demonstrated a marked difference in bactericidal power between citrated and hirudinized blood. This divergence points to some difference in action between citrate and hirudin—a difference which is explicable on the theory that hirudin contains no anti-thrombokinase, but is without an explanation if hirudin destroys thrombokinase and thus acts like citrate in preventing the formation of thrombin.

From the first series of experiments it is clear that blood which is allowed to clot in the slide-cell is superior in killing power to blood which has been prevented from clotting by the addition of sodium citrate. In the second series of experiments the blood which was prevented from clotting by the addition of hirudin was more bactericidal than the citrated blood, the difference between the two samples being due to the fact that citrate prevented clotting in the preliminary stages, whilst hirudin allowed the stages to proceed to the formation of thrombin.

Again, from the third series of experiments it appears that the longer blood remains after shedding before it is citrated the more bactericidal it becomes. In such blood thrombokinase is being liberated from the breakdown of platelets as the preliminary stages of clotting proceed.

In blood citrated just prior to clotting, where the bactericidal power is greatly increased, the greatest amount of thrombokinase will be present. Now the initial stages of clotting must begin in blood, however rapidly the blood is removed from a vein and citrated, and thus the bactericidal power will have increased. Accordingly the bactericidal power of blood as measured in a slide-cell is not an absolute measure of the bactericidal power of circulating blood.

CONCLUSION.

Blood develops bactericidal properties during the preliminary stages of clotting which bring about the formation of thrombin.

REFERENCE.

WALSH, V. G., AND HARMSWORTH, DAPHNE.—(1926) *Brit. J. Exper. Pathol.*, **7**, 129.

IMMUNITY IN EXPERIMENTAL HERPES.

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THE literature of experimental herpes provides abundant evidence that recovery from infection with the virus of herpes febrilis is accompanied by the development of a general immunity to reinfection with this virus. It is true that the degree of immunity may vary. In this the site and extent of the lesion would appear to play some part, and, as in all animal experiments, individual variation has to be expected and reckoned with. However, the statement that a considerable degree of general immunity is developed would appear to be subscribed to by all those who have studied experimental herpes. This unanimity goes no further, for when the mechanism of the immunity is in question, there is a considerable divergence of view. Levaditi (1926) is of the opinion that it is purely a tissue immunity, not being accompanied by the appearance of demonstrable antibodies in the blood, and this view is shared by Doerr and Vöchting, Blanc, Tsiminakis and Caminopetros, Luger and Lauda, Perdrau and others.* In support of this contention Levaditi and Nicolau (1922) advance experimental evidence to show that the tissues of animals immune to the virus of herpes have the power of neutralizing this virus *in vitro*. Perdrau (1925) failed to confirm this; in fact he found that emulsions of brain from herpes-immune rabbits, when incubated with herpes

* The reader is referred to the monograph 'L'herpès et le Zona' by Levaditi (1926), where reference to the papers on this question will be found.

virus, instead of neutralizing the virus exalted its virulence. This he attributed to the presence of an aggressin. Flexner and Amoss (1925) were able to demonstrate neutralizing power in the serum of rabbits recovered from an infection with herpes, but their experiments failed to convince because of the enormous quantities of serum—9 parts of undiluted serum to 1 part of virus—used in their neutralization experiments. More recently Zinnser and Fei Fang Tang (1926) have re-investigated this question, and find that though emulsions of brain from herpes-immune rabbits have some power of neutralizing the virus *in vitro*, the serum is similarly endowed and to a considerably greater degree. During the last few months an investigation of the herpes and allied viruses has enabled us to make some observations on this problem of immunity in experimental herpes, and the discordant views expressed in the literature of this subject have encouraged us to place our findings on record.

EXPERIMENTAL WORK.

In the study of the herpes virus we have made almost exclusive use of the guinea-pig as the experimental animal. Perhaps it might not be out of place to describe briefly the experimental disease in this animal before proceeding to the observations on immunity.

Experimental Herpes in the Guinea-Pig.

Although the susceptibility of the guinea-pig had been previously demonstrated by many investigators (Doerr and Vöchting, Luga and Lauda, Blanc and Caminopetros, Levaditi and Harvier, Goodpasture and Teague), it was Gildermeister and Herzberg (1925) who showed that the guinea-pig could be infected with the virus of herpes by inoculating it in the plantar skin. A local vesicle resulted, appearing about the third day, which healed slowly and often resulted in considerable scarring. No generalized lesions were ever encountered. This was confirmed (Bedson, Maitland and Burbury, 1927), and in the present investigation the suitability of the guinea-pig for experimental work with this virus has been amply borne out. There is no difficulty in infecting these animals with the fluid from fresh human herpes vesicles. Inoculated intradermally in the plantar skin, the sole of the foot appears hot, red and swollen in two days, and by the third day vesicles appear as whitish areas along the course of the inoculation tracks. These vesicles soon coalesce to form a single large vesicle occupying the major portion of the sole of the foot. The contents of the vesicle rapidly become frankly purulent. In some instances, particularly in the first transfer of human material to the guinea-pig, the vesicles may not appear before the fourth or fifth day. With subsequent passage, however, the incubation period becomes reduced to three days, and thereafter the virus behaves with marked regularity. Material for passage can be obtained by either evacuating the vesicles and taking up their content in a suitable diluent, or killing the animal and removing the vesicle epithelium. The latter can then be cut up fine, ground with sand and suitably diluted. The diluent employed has been an M/50 solution of K_2HPO_4 and KH_2PO_4 of a pH of 7·6.

Although this method of inoculation is satisfactory, a better procedure,

perhaps, is to rub the virus into the scarified and epilated hairy skin. The vesicles appear earlier, by the second day after inoculation, the number of vesicles depending on the dilution of the virus. Material for passage can be readily obtained without sacrificing the animal, and a further advantage in this mode of infection is that it lends itself to a ready titration of the virus We shall shortly refer to this question of titration in some detail, as we consider it to be of the greatest importance. Naturally immune guinea-pigs are rare; we have met with two out of some 300 animals inoculated. No generalization has ever been observed,* and when the disease has been allowed to run its course, complete recovery has been the invariable rule. Although other routes of infection have not been tried by us, the guinea-pig can be successfully inoculated intra-cerebrally and by scarification of the cornea (Remlinger and Bailly, 1926).

Titration of the Herpes Virus.

This has been carried out as follows: The vesicle epithelium is weighed, ground with sand, and then taken up with that quantity of diluent required to give a 10% suspension of the ground vesicle epithelium. The suspension is allowed to sediment in the ice-chest for one to two hours, or lightly centrifuged and the supernatant fluid pipetted off. This constitutes the 1 in 10 dilution of the virus and from it a series of dilutions is made. A guinea-pig is prepared by epilating two or three areas 2 cm. × 3 cm. on each flank. The skin of these epilated areas is then cleaned with alcohol, scarified with a sterile needle and the dilutions of virus rubbed in, a separate dilution to each area. The result is read in 48 to 72 hours, and the titre of the virus is taken as being the highest dilution which produces vesicles. It was soon found that with the strains of virus in use, a take was rarely observed above a 1 in 1000 dilution, so that as a routine procedure the titration is not taken above that figure, and although in some cases this results in the titration having no apparent end-point, the size of the lesion given by the 1 in 1000 dilution leaves little doubt that the end-point has actually been reached. Some examples of the results of titration of herpes virus are given in the following table:

TABLE I.

Virus.	Strain.	Dilutions of virus. 1/20.	1/100.	1/500.	1/1000.	Titre.
"Bowles"	B. 77	+++	++	+	±	1 in 1000
,,	B. 81	++	+	±	−	1 in 500
,,	B. 103	+++	++	+	±	1 in 1000
,,	B. 164/165	+++	+++	++	+	> 1 in 1000
"Barnett"	B. 210	+++	+++	++	−	1 in 500
"Crawley"	B. 233	+++	++	+	−	1 in 500
"J. G. C."	B. 240	+++	+++	++	+	> 1 in 1000
,,	B. 245/247	++	+	±	−	1 in 500
,,	B. 278	+++	++	++	±	1 in 1000
,,	B. 292/293	+++	++	+	±	1 in 1000

+++ = Confluent vesicles. ++ = Numerous non-confluent vesicles. + = Moderate number of vesicles. ± = Few vesicles.

* Since writing this paper two cases have been observed where spread to the cord occurred after intradermal inoculation in the plantar skin. Both guinea-pigs developed paralysis of the hind limbs and showed a meningo-myelitis of the lumbo-dorsal section of the cord.

It will be seen with what comparative regularity the titration works, and although, of course, no great degree of accuracy is claimed for the method, the results are sufficiently exact to enable work on a quantitative basis to be undertaken.

Time of Onset and Duration of Immunity.

Having ascertained that recovery from infection with herpes virus in the guinea-pig was associated with a considerable degree of general immunity, and further that the various strains of herpes febrilis cross-immunized—a point already established by other workers in this field—some observations on the time of onset and duration of immunity were next made. The results are tabulated below (Table II).

TABLE II.

Guinea-pig.	Site of first inoculation.	Interval.	Site of test inoculation.	Result of test inoculation.	
B. 94	Hairy skin	3 days	Feet	Typical vesicles.	Not immune.
B. 95	,,	5 ,,	,,	,,	,,
B. 86	,,	7 ,,	,,	*Nil.*	Immune.
B. 85	,,	7 ,,	,,	,,	,,
B. 84	,,	9 ,,	,,	,,	,,
B. 82	,,	9 ,,	,,	,,	,,
B. 42	,,	12 ,,	,,	,,	,,
B. 56*	,,	15 ,,	,,	,,	,,
B. 44	Feet	25 ,,	,,	,,	,,
B. 28	,,	44 ,,	,,	,,	,,
B. 21	,,	23 ,,	,,	,,	,,
,,	,,	23 ,,	Hairy skin	,,	,,
,,	,,	34 ,,	Feet	,,	,,
,,	,,	108 ,,	,,	,,	,,
B. 7	,,	43 ,,	,,	,,	,,
,,	,,	130 ,,	,,	,,	,,

It will be seen that immunity is present by the seventh day after inoculation, and further that the immunity is general, since inoculation on the hairy skin produces immunity of the plantar skin and *vice versâ*. Although the results obtained in the case of guinea-pigs B. 21 and B. 7 cannot be taken as clean experiments on the question of the duration of immunity, it is obvious that the immune state is not ephemeral.

The Presence of Neutralizing Power in the Serum of Herpes-immune Guinea-pigs.

This was the next point investigated, and the technique of the test was as follows: The sample of herpes virus with which the test was to be carried out was first of all titrated, and that dilution representing 50 minimal infecting doses determined. In the majority of cases this was a 1-in-20 dilution.

Dilutions of the serum were then made, and equal parts of the serum dilutions and diluted virus placed in small tubes. After standing at room temperature for 30 minutes, these mixtures were inoculated to guinea-pigs

* Only one vesicle produced.

by rubbing them into the scarified epilated hairy skin. In the first neutralization experiments the serum of the convalescent guinea-pigs was used undiluted, no attempt being made to determine the quantity of neutralizing antibody present. Having ascertained that these antibodies did occur quite constantly, the test was carried out quantitatively in subsequent experiments. Some examples of the results obtained are given below (Table III).

TABLE III.

Guinea-pig.	Time of collection of serum after first inoculation.	Titre of virus.	Dilution of virus used.	Dilution of serum.	Result.
B. 114	41 days	1 in 1000	1 in 20	1 in 1	Neutralized.
B. 7	72 ,,	,,	,,	,,	,,
,,	127 ,,	,,	,,	,,	,,
B. 21	34 ,,	,,	,,	,,	,,
,,	34 ,,	,,	,,	1 in 5	,,
,,	34 ,,	,,	,,	1 in 10	Partial neutralization.
,,	34 ,,	,,	,,	1 in 50	No neutralization.
Normal	—	,,	,,	1 in 1	,,
,,	—	,,	,,	,,	,,
,,	—	,,	,,	,,	,,
,,	—	,,	,,	,,	,,

From these examples it is quite clear that the serum of normal guinea-pigs fails to neutralize the herpes virus *in vitro*, whereas the serum of herpes-immune guinea-pigs is possessed of this faculty in considerable degree. It might be argued that the guinea-pig was not highly susceptible to this virus—less so than the rabbit, for example—and that this relative insusceptibility exaggerated a low neutralizing power of convalescent sera. That this is not so is clearly shown by the following parallel series of tests made on both guinea-pig and rabbit, the results in the two being identical (Table IV).

TABLE IV.

Animal.	Time of collection of serum after first inoculation.	Titre of virus.	Dilution of virus used.	Dilution of serum.	Result.	
					In guinea-pig.	In rabbit.
Guinea-pig B. 7	127 days	1 in 1000	1 in 20	1 in 1	Neutralized	Neutralized.
Guinea-pig B. 114	41 ,,	,,	,,	,,	,,	,,
Rabbit RB. 9	99 ,,	,,	,,	,,	,,	,,
—	—	,,	1 in 40	—	Numerous vesicles	Numerous vesicles.

In this table the result of a test carried out with the serum of a rabbit is included. This sample was collected 99 days after inoculation by corneal scarification, and here again neutralizing power is evident. The control inoculations made with virus diluted 1 in 40 show that a strain of herpes virus adapted to the guinea-pig by passage is still capable of producing a vesicular eruption in the rabbit when rubbed into the scarified epilated skin.

Time of Appearance of Neutralizing Antibody in Blood.

It has already been seen that general immunity was apparent by the seventh day after inoculation, and since this state has been shown to be accompanied by the presence of a neutralizing antibody in the serum, it became of interest to know when, in the development of the immune state, this antibody could be first demonstrated. Samples of blood were obtained from two guinea-pigs by cardiac puncture. They were then inoculated intradermally in the plantar skin with herpes virus. Typical lesions developed by the third day. Further samples of blood were collected on the seventh and ninth days after inoculation and the neutralizing power of these various samples of sera determined. The results are given in the following table (Table V):

TABLE V.

Guinea-pig.	Time of collection of serum.	Titre and dilution of virus employed.	Dilution of serum.	Result.
B. 313	Before inoculation	1/1000 1/20	1/1	No neutralization.
„	7 days after inoculation	1/500 1/10	„	Partial neutralization.
„	Ditto	„ „	1/5	No neutralization.
„	9 days after inoculation	„ „	1/1	Neutralization.
„	Ditto	— —	1/5	„
B. 314	Before inoculation	1/1000 1/20	1/1	No neutralization.
„	7 days after inoculation	1/500 1/10	„	„
„	Ditto	„ „	1/5	„
„	9 days after inoculation	„ „	1/1	Neutralization.
„	Ditto	„ „	1/5	„

Neutralizing power appears in the serum somewhere between the seventh and ninth day after inoculation—about the same time that general immunity can first be demonstrated.

Increase of Neutralizing Power by Hyper-immunization.

This possibility was next investigated, and from the results tabulated below it is obvious that a series of inoculations of herpes virus given to guinea-pigs convalescent from a herpes infection is capable of raising the neutralizing power of the serum. The "titre" of the serum referred to in this table is the highest dilution of serum capable of neutralizing 50 M.I.D. of virus.

TABLE VI.

Guinea-pig.	Titre of serum before hyper-immunization.	Details of hyper-immunization.	Titre of serum after hyper-immunization.
B. 212	1 in 2	Five inoculations s.c. with herpes virus as follows:	1 in 200
		11 . 10 . 26. 0·25 c.c. 1/20 virus	
B. 213	1 in 20	13 . 10 . 26. 0·25 „ „	1 in 200
		15 . 10 . 26. 0·5 „ „	—
B. 214	1 in 10	20 . 10 . 26. 0·5 „ „	1 in 20
		27 . 10 . 26. 0·5 „ „	—
B. 215	1 in 2	8 . 11 . 26. Test bleeding.	1 in 40

Neutralizing Power of Tissues of an Immune Animal.

Earlier in the paper reference was made to experiments of Levaditi and Nicolau, showing that the tissues of rabbits (brain), immune to the herpes virus, had the power of neutralizing that virus *in vitro*. On these experiments, and on their inability to demonstrate antibodies in the serum of herpes-immune rabbits, they based their hypothesis of tissue-immunity. It was also recalled that Perdrau (*loc. cit.*) was unable to confirm these observations; in fact, in his experiments emulsions of brain from herpes-immune rabbits, when incubated for 1 to 5 hours at 37° C. with herpes virus, instead of neutralizing the virus increased its virulence. An attempt was therefore made to see whether the tissues from guinea-pigs, immune to herpes virus, were able to neutralize the virus *in vitro*. Convalescent guinea-pigs, which had been tested and found immune to herpes virus, were bled by cutting the vessels in the neck. The blood was kept and the serum collected. Portions of hairy skin, previously epilated, and of plantar skin were excised, weighed, ground up with sterile sand and suspended in M/50 phosphate to give a 10% suspension of tissue. Similar preparations were made with normal guinea-pig tissue. Equal parts of serum or tissue suspensions were mixed with a given dose of herpes virus (50 M.I.D.), allowed to interact for one hour at room temperature, and then tested for the presence of active virus by rubbing into the scarified epilated skin of guinea-pigs. The results obtained are given below in the form of a table (Table VII).

TABLE VII.

Guinea-pig which supplied the tissues.	Equal parts of 10% tissue suspension or undiluted serum and herpes virus (50 M.I.D.) allowed to interact for 1 hour at room temperature.		
	Plantar skin.	Hairy skin.	Serum.
B. 251a. Herpes immune .	+++ .	+++ .	−
B. 317 „ .	+++ .	++ .	−
Normal	++ .	Not tested .	Not tested.
„	+++ .	„ .	++

+ + + = Confluent take.
+ + = Numerous non-confluent vesicles. } No neutralization.
− = No take. Neutralization.

These experiments are few in number, but the answer they give is quite clear. The tissue emulsions made from immune guinea-pigs had no neutralizing power for the herpes virus, whereas the serum in both cases had. In the case of normal guinea-pigs neither tissue-emulsions nor serum neutralized the virus. It is true that in these experiments the tissue-emulsions were left in contact with the virus for one hour only at room temperature, whereas in the experiments of Levaditi and Nicolau the mixtures were incubated for several hours at 37° C. and then placed in the ice-chest overnight before making the test inoculation. But it has been shown that in the case of the viricidal action of the serum, only a short contact at room temperature is necessary to demonstrate its action, and presumably the experiments of Levaditi and Nicolau were planned to show the presence of some viricidal substance in the

cells of the immune tissue, so that one would have expected that its presence would have been revealed in our experiments had it been present. It seems to us, however, that a tissue immunity, as apart from a humoral immunity, would be more likely to depend on some intrinsic change in the cells themselves only to be demonstrated in the intact cells *in situ*, and not capable of demonstration when these cells had been removed from the body and ground up.

Passive Immunity.

The possibility of increasing the neutralizing power of the serum of herpes-immune guinea-pigs by a process of hyper-immunization led us to investigate the question of passive immunity. The following experiment was carried out: Two guinea-pigs were given intra-peritoneal inoculations of 20 c.c. hyper-immune serum. Twenty-four hours later these two animals, together with a normal one (all three were of the same size—approximately 400 gm.), were used for the titration of strain of herpes virus as described above. The results are given below, and clearly show that the inoculation of the hyper-immune serum reduced considerably the receptivity of the two guinea-pigs to herpes virus.

TABLE VIII.

Dilutions of virus.	*Guinea-pig B.* 300. 2·0 c.c. serum B. 212. I.P. 24 hours before inoculation.	*Guinea-pig B.* 301. 2·0 c.c. serum B. 213. I.P. 24 hours before inoculation.	*Guinea-pig B.* 303. Normal control.
1 in 20	Poor take. Seven vesicles	Poor take. Twelve vesicles	Confluent take.
1 in 100	No take	Very poor take. Two vesicles	Numerous vesicles becoming confluent.
1 in 500	Not tested	Not tested	Moderate take.
1 in 1000	„	„	No take.

In vitro *Tests with Herpes Virus and Herpes Anti-sera.*

Agglutination-precipitation.—Numerous attempts were made to demonstrate the interaction of herpes virus and its specific antibody, using both convalescent and hyper-immune guinea-pig sera. The various antigens tried were:

(1) Saline suspensions of guinea-pig herpes vesicle epithelium employed in different strengths, and made from both fresh vesicle epithelium and from material which had been stored in 50% glycerol in the cold.

(2) Alcoholic extract of guinea-pig herpes vesicle epithelium:

(*a*) Different concentrations in saline.

(*b*) Different concentrations in saline with the addition of an alcoholic extract of benzoin (Dujarric de la Rivière, 1923).

No success attended the use of any of these antigens either as a flocculation or a ring test.

Complement-fixation.—Here again our efforts were fruitless. Both saline suspensions and alcoholic extracts were tried, and though in some cases there was evidence of specific fixation, the results were never clear-cut.

It is recognized that our efforts in this direction were not exhaustive, and the excellent results obtained by Gordon (1925) in the case of vaccinia

(agglutination and complement-fixation) and by Kraus and Takaki (1926) with rabies (complement-fixation) would point to the same being possible in the case of herpes. Most probably it is a question of finding a suitable antigen, but as the neutralization experiment had given such satisfactory results in our hands and was so simple to perform, we decided to abandon our search for a satisfactory *in vitro* test.

SUMMARY.

It has been shown that recovery from infection with the virus of herpes in the guinea-pig is accompanied by the development of a general immunity to this virus, and that the serum of these convalescent guinea-pigs possesses the power of neutralizing the herpes virus *in vitro*. Further, it has been found possible to increase this viricidal activity of the serum by hyper-immunization, and with the hyper-immune sera so obtained to produce a definite degree of passive immunity. We have not been able to demonstrate that tissue emulsions from herpes-immune guinea-pigs possessed any neutralizing power for the herpes virus *in vitro*, but would not, therefore, on that score rule out the possibility of a tissue immunity apart from a purely humoral one. It would appear, then, that there is nothing peculiar about the mechanism of the immunity in experimental herpes. As in other immune states, whether to filterable viruses, such as vaccinia, or to bacteria, one has to consider the two factors, the cells and the humours, both acting together to ensure the immune state. The relative importance of these two factors undoubtedly varies in the immunity developed to the different viruses—using the term "virus" in its broadest sense—and the importance of the *rôle* of the viricidal or neutralizing power of the blood-serum in experimental herpes is open to argument. However, it would appear incorrect to state that immunity to the virus of herpes is purely a tissue immunity, and to dismiss humoral immunity as non-existent or entirely negligible.

The findings of Flexner and Amoss, and of Zinnser and Fei Fang Tang, which have been confirmed and extended by us, show clearly that such an attitude is unwarranted. In conclusion, we would suggest that the probable reason for the failure to demonstrate the existence of these neutralizing antibodies in the herpes-immune state lies in the fact that the problem has not been approached quantitatively.

We wish to take this opportunity of expressing our indebtedness to the members of the Clinical Staff of the London Hospital, more particularly those of the Medical Unit and the Department of Dermatology, who have so kindly supplied us with cases of herpes.

REFERENCES.

Bedson, S. P., Maitland, H. B., and Burbury, Y. M.—(1927) Second Progress Report of the Foot and Mouth Disease Research Committee (Appen. II). London (H.M. Stationery Office).

Dujarric de la Rivière.—(1923) *C.r. Soc. Biol.*, **89**, 1198.

Flexner, S., and Amoss, H. L.—(1925) *J. Exp. Med.*, **41**, 357.

GILDERMEISTER, E., AND HERZBERG, K.—(1925) *Deut. med. Woch.*, **51**, 97.
GORDON, M. H.—(1925) *Med. Res. Counc. Spec. Rep. Series*, No. 98. London (H.M. Stationery Office).
KRAUS, R., AND TAKAKI, J.—(1926) *Wien. klin. Woch.*, **39**, 624.
LEVADITI, C.—(1926) 'L'herpes et le Zona.' Paris (Masson et Cie).
LEVADITI, C., AND NICOLAU, S.—(1922) *C.r. Soc. Biol.*, **86**, 228.
PERDRAU, J. R.—(1925) *Brit. J. Exp. Path.*, **6**, 41.
REMLINGER, P., AND BAILLY, J.—(1926) *Ann. Inst. Past.*, **40**, 253.
ZINNSER, H., AND FEI FANG TANG.—(1926) *J. Exp. Med.*, **44**, 21.

A TRANSPLANTABLE FIBRO-SARCOMA OF THE FOWL.

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TUMOURS of fowls are relatively very common and comprise a great variety of histological types—sarcomata, carcinomata, fibromata, myomata and lymphomata.

The tumour to be here described arose in a white Leghorn fowl, and was discovered at post-mortem examination on August 11th, 1925. This hen had been ill for some days and had to be killed. No cause for its illness was seen until a hard whitish tumour, 4·5 × 2 cm., was found in the mesentery about a loop of intestine and apparently causing obstruction. It was removed as aseptically as possible. No metastases were seen. On section it was found to be very hard, and the cut surface showed the same pearly-white appearance as the external surface, but with yellower areas here and there which were also firm and hard.

Five young white Leghorn fowls were inoculated with small grafts of 0·015–0·02 gm. into the muscles on the right side of the sternal keel. One of these birds was missing on September 10th. The tumour grew slowly in the four remaining birds, and on October 2nd, *i.e.* 52 days later, it was re-transplanted. The tumour was then approximately 4 × 2 cm. Altogether it has now been transplanted four times. The small number of transplantations that have been required to keep the tumour going over a period of 18 months will give some idea of its slow rate of growth.

The third transplantation done on March 1st, 1926, was from one of the chicks first inoculated. This chick, which had been inoculated 202 days previously, had a huge tumour extending from well above the anterior end of the keel to well below, a distance of about 15 cm.; in breadth it was about 8 cm. On opening the bird it was found that the tumour bulged into the peritoneal cavity. Notwithstanding the size of the growth and the time it had been growing there were no metastases found anywhere, though they

were carefully searched for. This enormous growth was hard to the touch, and on section showed the same appearance, as did the original, viz. a hard pearly-white growth with yellower areas throughout. On November 1st, 1926, one of the birds from the previous inoculation eight months before had to be killed. It had a large growth projecting not only externally, but internally also. As before, it was extremely hard, and there were no metastases.

Two birds were inoculated, but up to the present have no palpable growths.

On February 12th, 1927, a bird which had been inoculated on March 19th, 1926, *i. e.* eleven months previously, was so burdened by the weight of the tumour it had to carry that it had to be killed. The huge growth projected internally into the thorax and abdomen. The interesting and new point, however, is that the left lung was almost replaced by a metastasis, while another was found in the mesentery. No connection could be found between the projecting mass of tumour into the body and these growths, which are therefore regarded as true metastases. In both cases they were like the primary growth—white and extremely hard.

It does not appear to grow so well in birds of a different strain, as a Plymouth Rock chicken inoculated by Dr. Gye was kept for 250 days, at the end of which time the growth was only a little larger than a walnut.

Structure: In every case the macroscopic and microscopic appearance of the tumour has been the same. Microscopically one finds dense bands of fibrous tissue.

DISCUSSION.

The microscopic appearance, the slow rate of growth and the rarity of metastases all indicate that we are dealing here with a relatively benign transplantable fibro-sarcoma of the fowl.

In 1922 we had in the laboratory of the Imperial Cancer Research Fund a similar tumour to the one here described. This arose in a white Leghorn hen and was of enormous size, extending all over one side of the bird.

It was transplanted and grew for one or two generations and then died out. In consistency, slow rate of growth and microscopic appearance it was similar to the present tumour.

So far, owing to the extremely slow rate of growth, no attempts have been made to grow the tumour by filtrates or cultures, *i. e.* apart from living cells.

SUMMARY.

A slow-growing fibro-sarcoma of the fowl has been propagated with living cells for four generations.